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Ph.D. thesis

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Denmark, July 2007

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Pictures on front: The growth system used in experiment 1

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Denmark, July 2007

Preface

The present thesis is submitted, as the written part required to obtain the Ph.D. degree at the University of Copenhagen. The work was carried out at the Department of Horticulture, University of Aarhus under supervision of Kristian Thorup-Kristensen and Eva Rosenqvist, and at the Department of Agricultural Sciences, University of Copenhagen under supervision of Jesper Mazanti Aaslyng. The work was financially supported by Faculty of Agricultural Sciences at the University of Aarhus (formerly known as the Danish Institute of Agricultural Sciences).

First, I would like to thank my supervisors, Kristian Thorup-Kristensen, Eva Rosenqvist and Jesper Mazanti Aaslyng. They have all been great inspirators, and have always made time for me. Further, I would like to thank Carl Otto Ottosen, for encouragement and constructive discussions during the work. Kaj Ole Dideriksen, Ruth Nielsen, Helle Sørensen and Connie Damgaard are thanked for skilful technical assistance, and special thanks to Lene Korsholm Jørgensen who always placed me in front of the line for the HPLC. Thanks to Ina Hansson, who provided me with company during part of the experiments, and challenged me on my knowledge about plant physiology.

Thanks to Hanne Lakkenborg Kristensen, for providing me with knowledge about ^{15}N-nitrate, and Majken Pagter, for fruitful discussions about much more than just science. At last, but not least, the best husband Anders Kjær, for his endless support and patience.

This thesis contains five chapters, three manuscripts and a reference list: Chapter one is a general introduction to the subject of this thesis. Chapter two and three contains literature reviews and the main questions asked. Chapter four contains an introduction to the methods and the experimental work carried out in the form of three manuscripts, placed after chapter five. Chapter five contains conclusions and perspectives.

Katrine Heinsvig Kjær, July 2007

Table of contents

Summary

The main objective of the studies described in the present Ph.D. thesis was to investigate the effect of low night temperatures on plant physiological responses, and to discuss this knowledge in relation to an optimisation of dynamic climate control systems in greenhouse production of floricultural crops.

Dynamic climate control systems optimise greenhouse production of plants in order to save energy. The temperature and carbon dioxide (CO_2) concentration in the air of the greenhouse, are controlled according to the natural variation in irradiance, and allowed to vary considerably more than in "standard" climate control programmes for greenhouse production. While the system is mainly based on the understanding of photosynthesis, the potential for energy saving is based mainly on the ability to reduce temperatures, when the irradiance is low, and during the night, without reductions in plant growth. Our understanding of plant reactions to low night temperatures is limited. While growth in the sense of carbon gain and biomass production stops when there is no light, growth is maintained, in the sense of cell division and elongation, nutrient uptake, and transport of carbohydrates and nutrients. These processes are all energy-consuming and temperature-dependent, and therefore reduced night temperatures may change source-sink relations in the plants and delay plant development well before direct effects are seen on dry matter (DM) production and plant size.

Three experiments were performed to show how plants mobilise and utilise energy in terms of nutrients and carbohydrates during the night, and at low night temperatures. The plant chosen for the experiments was *Chrysanthemum x morifolium* L. cv. 'Coral Charm'. In the first two experiments (Paper I and II), plants were grown in water-based nutrient solution cultures, in climate chambers. This was done to eliminate natural fluctuations in the light and temperature. Water was used instead of peat, to eliminate possible effects of temperature on nutrient transport in a heterogeneous growth substrate.

In the last experiment (Paper III), plants were grown in peat and in a greenhouse, with the purpose of extrapolating the results from the controlled environments in the climatic chambers to a production site, where plants were also subjected to the influence of reduced night temperature in combination with external factors, such as nutrient transport in peat and humidity of the air. Plants were grown in long day conditions in all three experiments, to ensure that plants remained vegetative, as chrysanthemums are known to flower at short day conditions with a photoperiod of less than 12 h.

During the experiments an analysis of the overnight plant NO_3^- uptake was performed with the use of the stable isotope ^{15}N. The $Na^{15}NO_3^-$ was applied to the growth substrate just before the dark period and excess ^{15}N content was measured after the dark and light period, respectively. Diurnal and long term changes in carbohydrate levels in leaves and roots were studied in conjunction with studies of plant growth, including root growth and morphology.

In Paper I, it was shown that low night temperatures down to 8ºC did not affect overall plant dry matter (DM) production and NO_3^- uptake of *Chrysanthemum x morifolium*; however, plant morphology was changed and starch accumulation occurred in the leaves. It

was concluded, that the main limitation to chrysanthemums grown at a low night temperature was sink limitation. Assimilates from the photosynthesis remained in the leaves as starch, on the expense of an investment in leaf initiation and expansion.

In Paper II, root zone heating was applied to the plants in order to study the hypothesis that root zone heating is a beneficial tool to overcome negative effects on plant growth and physiology, when plants are grown at low night temperatures. However, root zone heating did not decrease starch accumulation, which rejected the hypothesis that increased root growth and activity of heated roots would provide a larger sink for carbohydrates and increase the carbohydrate export and decrease the starch accumulation in the leaves.

In Paper III, an experiment was performed under fluctuating light and temperature conditions in a greenhouse, in order to study whether it was possible to grow chrysanthemums at LNT, without a loss in dry matter (DM) production or significant changes in morphology. Low night temperatures increased starch accumulation in the leaves, but not as much, as in the climate chamber experiment, and the low night temperatures did not have major influences on plant morphology and plant growth. It was concluded, that it is possible to grow chrysanthemums at lower night temperatures, than what is normally used in greenhouse production of this crop.

From the present results, it is suggested to include knowledge about source-sink relations in plants in the future optimisation of the dynamic climate control system. It is suggested that the night temperature needs to be balanced in relation to the temperature and irradiance of the preceding day, in order to obtain a balance between the carbohydrates assimilated in photosynthesis, and the ability of the plants to transport and use the carbohydrates.

Dansk resume

Formålet med denne ph.d. afhandling var at undersøge effekten af lave nattemperaturer på fysiologiske mekanismer i planter, og at diskutere denne viden i relation til en optimering af dynamisk klimastyring i væksthusproduktion af potteplanter.

Dynamisk klimastyring i væksthusproduktion af potteplanter gør det muligt at optimere planteproduktion og samtidigt spare energi. Tilførslen af CO_2 øges i perioder, hvor det er varmt, og hvor lysindstrålingen er høj, samtidigt med at temperaturen får lov til at stige til højere værdier end i et traditionelt klima. Systemet er hovedsageligt baseret på en forståelse af planternes fotosyntese, men hovedparten af energien er sparet i perioder hvor lysindstrålingen er lav og om natten.

Vores forståelse af planters respons på lave nattemperaturer er begrænset. Vækst, i form af tørstofproduktion stopper når der ikke er noget lys, men vedligeholdelsesprocesser, i form af celledeling, strækningsvækst, næringsoptagelse og transport af næringsstoffer og kulhydrater forsætter. Disse processer kræver energi, og er temperaturafhængige. Derfor forventes det, at lave nattemperaturer ændrer på plantens source/sink balance og forsinker udviklingen, længe før plantens vækst synligt reduceres.

Der blev udført tre eksperimenter med henblik på at øge forståelsen af, hvordan planter mobiliserer og udnytter energi i form af næringsstoffer og kulhydrater om natten, og ved lave nattemperaturer. *Chrysanthemum x morifolium* L. 'Coral Charm' blev brugt som modelplante. I de første 2 eksperimenter (Artikel I og II) blev planterne dyrket i vandbaserede næringsopløsningskulturer og i klimakamre. Dette blev gjort for at undgå kendte effekter af lave temperaturer på transporten af næringsstoffer i jord og andre heterogene vækstmedier, og for at isolere planternes respons på lave nattemperaturer fra andre klimafaktorer.

I det sidste eksperiment (Artikel III) blev planterne dyrket i tørv og i væksthus, med det formål at sammenligne resultaterne fra de kontrollerede forhold i klimakamrene med resultater fra det miljø, hvor planterne normalt bliver produceret kommercielt. I alle tre eksperimenter blev planterne dyrket ved lang dagslængde for at sikre at planterne forblev vegetative, da krysantemum initierer blomstring ved en lysperiode på mindre end 12 timer.

Den stabile isotop ^{15}N blev brugt til at analysere planternes NO_3^- optagelse om natten. Der blev tilført $^{15}NO_3^-$ til planternes vækstmedie lige før mørkeperiodens start og meroptaget af ^{15}N i planterne blev målt efter både mørkeperiode og efterfølgende lysperiode. Daglige ændringer, og ændringer over længere tid i kulhydratfordelingen i planterne blev studeret i sammenhæng med plantevækst og morfologi af både skud og rødder.

I Artikel I blev det vist, at nattemperaturer ned til 8ºC ikke havde nogen effekt på planternes tørstofproduktion og NO_3^- optagelse. Dog var planternes morfologi ændret, og der var en øget stivelseophobning i planternes blade. Konklusionen var at den mest begrænsende effekt på planternes udvikling ved lave nattemperatur var en begrænsning af planternes source/sink balance. Produkter fra fotosyntesen forblev i bladene som ophobet stivelse i kloroplasterne, på bekostning af en investering i ny bladdannelse og bladudvidelse.

I Artikel II blev det studeret om opvarmningen af rodnettet ved lave nattemperaturer havde en positiv effekt på planternes source/sink balance. Hypotesen var, at øget aktivitet i rødderne ville øge rodvæksten og behovet for kulhydrater, hvilket ville øge eksporten af kulhydrater fra bladene og mindske stivelsesophobningen. Hypotesen blev afvist, idet transporten ind i floemet formodentlig var hæmmet af den lave temperatur i bladene.

I Artikel III blev et eksperiment udført under naturlige svingninger i lys og temperatur i et væksthus. Formålet var at studere om det var muligt at dyrke vegetative krysantemums ved lave nattemperaturer uden at ændre på planternes tørstofproduktion og morfologi. Den lave nattemperatur øgede stivelsesophobningen i bladene, men ikke nok til at det havde en effekt på planternes vækst og morfologi. Konklusionen var at det er muligt at dyrke krysantemum ved lavere nattemperaturer, end hvad der normalt bruges i kommerciel produktion.

På basis af de præsenterede resultater foreslås det at inkludere viden om planternes source/sink balance ved forskellige nattemperaturer i den fremtidige optimering af dynamisk klimastyring. Det forventes at en bedre balance mellem nattens temperatur i forhold til den foregående dags temperatur og lysindstråling, vil forbedre balancen mellem den mængde kulhydrater planten har assimileret i fotosyntesen, med den mængde kulhydrater som planten kan transportere og udnytte.

1. General Introduction

The first chapter of this thesis is an attempt to briefly introduce the dynamic climate control system, in its relevance for growing floricultural crops at low energy costs. In chapter two, the literature concerning specific plant responses to low night temperatures (LNT) are reviewed with focus on the literature on floricultural crops. In chapter three, the current knowledge about carbohydrate metabolism and nitrate uptake in plants are reviewed, in order to discuss the relevance of these two processes in response to LNT. Chapter four includes and outline of the experimental work and a description of materials and methods. Chapter five is a conclusion and suggestions of future directions, based on all the results presented in this thesis. Three manuscripts are placed after chapter five. The references of the first chapters are presented in one list placed at the end of the thesis.

1.1 Background

In the recent years, it has proved feasible to implement dynamic climate control for some floricultural crops (Aaslyng *et al*., 2003). The temperature and carbon dioxide (CO_2) concentration in the air of the greenhouse, are controlled according to the natural variation in irradiance, and allowed to vary considerably more than in "standard" climate control programmes for greenhouse production, which tend to keep the temperature as constant as possible.

The system is based on a model of leaf photosynthesis, calculated as a function of irradiance, temperature and CO_2 concentration. From this model, the system generates a two-dimensional array of photosynthesis rates as a function of chosen temperatures and CO_2 concentrations at a random irradiance (Figure 1.1). The dynamic climate described in Aaslyng *et al*. (2003) is optimised in several steps. 1) Irradiance is measured at canopy level. 2) An array of photosynthesis rates are calculated on the basis of chosen temperatures and CO_2 concentrations. 3) The maximum photosynthesis is determined from the array of photosynthetic rates. 4) The lowest temperature and CO_2 concentration are determined in relation to the photosynthesis set point. For example leaf photosynthesis of 80% of maximum photosynthesis, as this has proven to give good quality plants with less energy consumption compared to 100% photosynthesis (Lund *et al*., 2006).

Plant production is generally maintained in the system in comparison with plant production in more traditional climates, indicating a beneficial outcome of saving energy in periods were plants are less active. During the night, the air temperature set point of the greenhouse is kept constant and at the lowest acceptable limit for the actual crop, 15ºC for the main part of crops studied until now. An optimisation of photosynthesis is obtained by applying a closer connection between the day temperature and the irradiance, by allowing the temperature to rise to 30ºC before ventilation, and by increased supply of CO_2 (Aaslyng *et al*., 2003). Lower energy costs in the production of *Capsicum annuum* (Bell peppers), and *Hibiscus rosa-sinensis* was obtained by using dynamic climate control (Ottosen *et al*.,

2003; Lund *et al.*, 2006); however, a careful management of the climate was needed, to prevent delays in production time, and a decrease in plant quality.

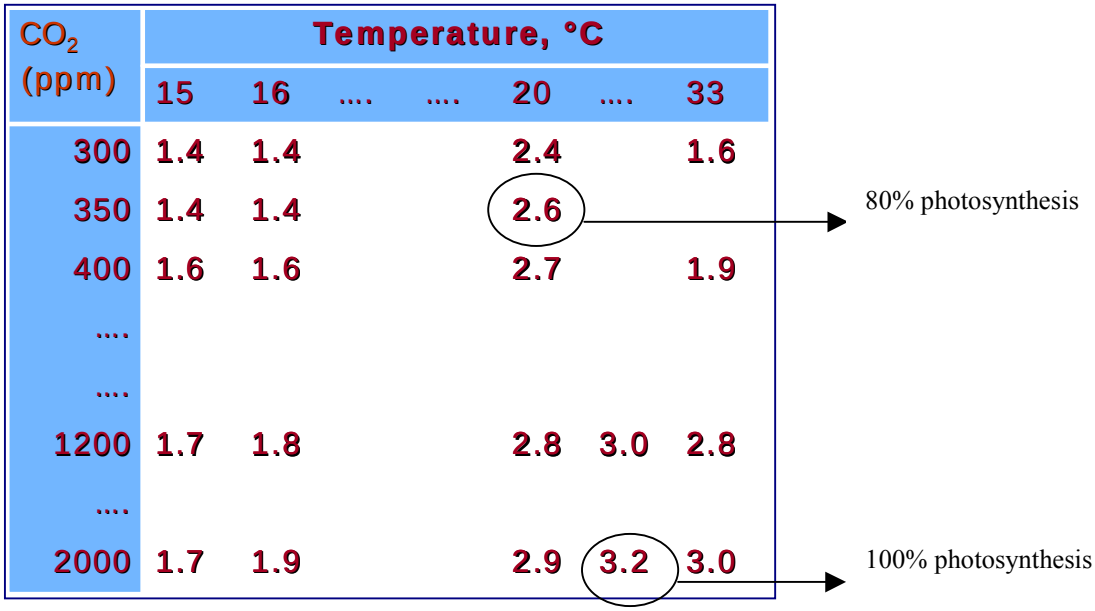

Figure 1.1
The index for percent of max photosynthesis at different temperatures and CO_2 concentrations at a random irradiance level. The largest number in the index refers to 100% photosynthesis Taken from Rosenqvist and Aaslyng (2000).

One of the greatest problems in producing floricultural crops at high day temperatures, in combination with low night temperatures, is the increased stem elongation due to the large difference in temperature between day and night. The phenomenon is known as DIF, and a positive increase in DIF, have been shown to increase plant height in many species (Erwin *et al*., 1989), and thereby increase the need to apply more chemical growth retardants to the crop (Körner, 2003). However, when plants of *Hibiscus rosa-sinensis* were grown under dynamic climate control, where the average positive DIF was higher in comparison with the control climate, plants became shorter (Lund *et al*., 2006). It is suggested, that the average positive DIF of the whole period, did not reflect the temperature difference between day and night of individual days in the period, because of large fluctuations in temperature. The results indicate that the concept of DIF does not hold in a dynamic climate, because of large temperature fluctuations within and between days. The results support the use of the dynamic climate control system in plant production.

The set points of minimum and maximum temperature in the dynamic climate control system are adjusted according to the acceptable temperature limits of the actual species grown in the system. In the experiments carried out at the Danish Institute of Agricultural Sciences (Now University of Aarhus, Faculty of Agricultural Sciences) and at The Royal Veterinary and Agricultural University (Now University of Copenhagen, Faculty of Life Sciences) in the years 1997 – 2002, more than one crop were grown in each system, and

therefore the temperature limits had to be a compromise between the different crops (Table 1.1). It is possible, that some crops can cope with a wider span in the temperature regime, which would increase the energy savings and optimise the system further. More knowledge in this area is needed, and it is believed that knowledge about the temperature limits of general plant physiological responses will provide more knowledge about the lower limits of night temperature and upper limits of day temperature, which a particular crop can cope with, without suffering from growth limitations.

The main objective of the studies described in the present Ph.D. thesis was to investigate the effect of low night temperatures on plant physiological responses, and to discuss this knowledge in relation to an optimisation of dynamic climate control in greenhouse production of floricultural crops.

Table 1.1
Overview of the dynamic control climates used in the experiments at the Danish Institute of Agricultural Sciences and at the Royal University of Veterinary and Agricultural Sciences in the years 1997 – 2002. Modified from Ottosen *et al*. (2005).

Treatment	Description
STD21/19	Standard climate 21/19ºC day/night temperature, 600 ppm CO_2
80%15	80% photosynthesis, 15ºC minimum temperature
90%15	90% photosynthesis, 15ºC minimum temperature
80%15DGT	80% photosynthesis, 15ºC minimum temperature, obtained with a DGT-volmatic climate computer
80%15(18)	80% photosynthesis, 15ºC minimum temperature (18º average minimum temperature)
80%17	80% photosynthesis, 17ºC minimum temperature
80%17TP	80% photosynthesis, 17ºC minimum temperature with short temperature peaks during the night
XF15Avg18	%photosynthesis regulated by avearage temperature

2. The influence of low night temperature on growth and development of floricultural crops

2.1 Introduction

Floricultural crops are cultivated plants with various origins ranging from the tropics to alpine and arctic regions. They are often crossbred across geographical barriers, growth types, and genus, to optimise growth and flower characteristics for production and marketing. It makes it difficult to predict the optimum growth conditions for each cultivar. Plants produced at tolerable, but non-optimal temperatures will continue to grow, but the result may be a longer production time, or a reduced quality. This chapter summarises the literature concerning the possibilities, and limits, in growing floricultural crops below their optimum temperature range during the night, but with optimal conditions during the day. When plants are grown below their optimum temperature during the night, they are often said to grow at low night temperature (LNT); however, there is no common agreement on what defines LNT, and how it differs between plant species and cultivars. In the work of this thesis, LNT is defined as temperatures below the optimum temperature range of a specific plant species or cultivar, but above temperatures at which plant growth is expected to stop, or to show large growth limitations.

The latest literature on producing floricultural crops at LNT is highly concentrated around cultivars of chrysanthemums with an optimal temperature range of 18 – 20ºC (van der Ploeg and Heuvelink, 2006). However, studies with other plants will be included, when available. The cost of keeping the night temperature, within the optimal temperature range inside the greenhouse, depend on the outdoor climate (wind and temperature), the insulation of the greenhouse (curtains and wall material), and off course the heating system. The energy cost is high in the cold period of the year, where the heating system is often required to keep the temperature above the minimum set point, and low in the warm period of the year, where temperatures above the minimum set point can be maintained by drawing the curtains, and by using the energy obtained from infrared irradiance of the sun. When night temperature is allowed to drop below the optimum temperature range for plant growth and development, plant growth will often become slower, and flower initiation and development may be delayed. However, this is not only a negative effect, as it can also be a cheap tool to control plant height and plant development in order to be able to deliver a certain product to the market at a certain time.

2.2 Plant growth

Biomass production

Night temperatures down to 10°C have been shown to increase the mean relative growth rate (RGR) of chrysanthemum cultivars grown at long day conditions (Table 2.1) (Parups and Butler, 1982); however, no increase in RGR was seen in other cultivars in the same study, and in *Chrysanthemum x morifolium*, when grown at a LNT of 12ºC and 8ºC in the

experiments of this thesis. RGR describes the rate of increase in plant dry matter (DM) per unit DM already present. Changes in RGR is often explained by changes in the rate of increase in the net assimilation rate (NAR, g cm^{-2} day^{-1}), or changes in the leaf area ratio (LAR, cm^2 g^{-1}). Differences in RGR across plants species, are explained mostly by a differentces in LAR, whereas the increase or maintained RGR in chrysanthemums grown at a LNT, is probably more an effect of an increase in NAR, caused by a lower maintenance respiration rate during the night as the CO_2 assimilation rate of chrysanthemums have been shown to decrease or remain unaffected in response to a LNT (Kohl & Thigpen, 1979; Kjær *et al*., Paper I).

Table 2.1
A short summary of the effects of low night temperature (LNT) on growth parameters in some floricultural crops. (+) illustrates an increase at LNT, (-) illustrates a decrease at LNT and (0) illustrates that LNT had no effect on the parameter. Several cultivars were often included in the studies, which explain why there is more than one effect of LNT in each study. The table is modified from van der Ploeg and Heuvelink (2006).

Crop and measurement	Night temperature treatments (control, low,)	RGR	Shoot weight	Biomass allocation Flowers	Leaves	Stems	Roots	No. of flowers	Time to flowering	Plant height
Chrysanthmum at flowering										
Parups and Butler, 1982	(16ºC, 16 and 10ºC)	+ 0	+ 0		+			0		
Tsujita *et al.* 1981	(16ºC, 13ºC)		+ 0	+ 0			0		+	+ 0 -
Kohl and Mor, 1981	(16ºC, 5ºC)		+ 0	0	+ 0	+ 0	+ 0	+ 0	+	0
Bonaminio and Larson, 1980	(16ºC, 16 and 10ºC)		+	+ -					+	+
Begonia at flowering										
Willumsen and Moe, 1995	(24ºC, 21ºC, 18ºC, 15ºC)		+ 0	0				0	+	+
Petunia at flowering										
Merritt and Kohl, 1989	(18ºC, 7ºC)		0	-				-	+	-
Geranium at flowering										
Merritt and Kohl, 1989	(18ºC, 7ºC)		-	-				0	+	-

While RGR is only reported in few publications, DM production of plant shoots is reported for a range of species grown at LNT. DM production of the plant shoot at flowering, has been shown to increase or remain unaffected at LNT, in cultivars of bedding plants and pot plants of roses, marigolds, begonia, hibiscus, petunia and chrysanthemum (Table 2.1)(Van

der Berg, 1987; Merrit and Kohl, 1991; Willumsen and Moe, 1995, Lund et al., 2006; Van der Ploeg and Heuvelink, 2006) or decrease in cultivars of certain bedding plants as petunia, geranium and impatiens at a night temperature of 7ºC (Merrit and Kohl, 1989, Merrit and Kohl, 1991). For chrysanthemums, the effects of LNT on final plant DM production at flowering are contradictory (Van der Ploeg and Heuvelink, 2006). The effect is possibly cultivar dependent, and dependent on interactions with other growth conditions, such as light intensity, and the length of the cultivation period. In the results shown in this thesis, it is further demonstrated that an increase in leaf DM in *Chrysanthemum x morifolium* cv. "Choral Charm" grown at a night temperature of 8ºC, in nutrient solution culture, and under climate chamber conditions, is explained in part by increased accumulation of starch in the leaves.

Biomass allocation

Biomass allocation is not very well-studied in floricultural crops. One explanation is that plant development, and the number of flowers and buds, are of more interest seen from a production aspect, than the actual weight of the organs. However, LNT or low temperature during part of the night, have been shown to increase DM production of stems and leaves, but not flowers, at flowering in chrysanthemums grown at short day conditions (Table 2.1) (Kohl and Mor, 1981; Parups and Butler, 1982; Karlson and Heins, 1992). Increased DM content of the leaves at LNT was explained by a delay in flowering, which increased the number of leaves formed beneath the flower (Karlson and Heins, 1992). A delay in flowering was also reported by Bonamino and Larson (1980); however, in that study, flowers produced at LNT were larger and heavier, than flowers produced at "normal" night temperatures. Larger and heavier flowers were also reported by Cockshull *et al*. (1967) and Carvalho *et al*. (2005). In roses, an increase in flower bud weight at LNT was reported by Van der Berg (1987). For begonia, LNT have been shown to reveal no changes in the biomass of leaves and flowers (Willumsen *et al*., 1995), whereas in bedding plants of petunia, LNT was shown to increase DM content of leaves on the expense of DM content of stems and flowers, and in geranium cultivars a decrease in plant DM production at LNT, corresponded to a decrease in DM content of both stems, leaves and flowers (Merrit and Kohl, 1989).

Knowledge about root growth at LNT is limited in floricultural crops. One reason is, that root studies are limited by the fact that the roots are hard to separate from growth substrates, such as peat and compost. In the results of this thesis, it was shown that the root DM decreased or remained unaffected by LNT in *Chrysanthemum x morifolium* grown in nutrient solution culture, and that root zone heating did not change this pattern (Kjær *et al*, paper II). It seems reasonable to conclude, that LNT increase the DM content of the above ground parts of some chrysanthemum cultivars. However, it is difficult to extrapolate this knowledge to other plant species as plant DM allocation depend on plant genotype, and in which way, different plants respond to various growing conditions.

Nutrient uptake

It is well-documented, that reduced root zone temperatures decrease the uptake and content of different nutrients in plants (Cooper, 1973), which makes nutrient uptake of floricultural crops an important issue. However, problems are largely overcome by changing the composition of the nutrient solution applied to the cultures, and maintaining pH (Stensvand and Gislerød, 1992). This is one reason, why nutrient uptake studies at LNT are scarce in floricultural crops. Another reason is that nutrient transport within plants during the night, is believed to be of minor importance in relation to overall nutrient uptake, plant growth and development, because xylem transport of nutrients to the shoot is related to transpiration, which mainly occur during the day (Rufty *et al*., 1984).

Increased nutrient contents have been shown to increase plant longevity in potted poinsetta (Scott *et al*., 1989), and in roses (Mortensen *et al*., 2001). Furthermore, the number of roots formed by cuttings of chrysanthemums and pelargoniums have been shown to be positive correlated to nitrogen availability of the stock plants (Druege *et al*., 2000, Druege *et al*., 2004). In these studies differences in plant nutrient concentrations were obtained by exposing plants to different combinations of nutrient solutions. However, abiotic factors such as increased CO_2 concentrations and low temperatures also change the amount of nutrients in the plant tissue (Kuehny *et al*., 1991; Trusty and Miller, 1991; Kjær *et al*., Paper I). A large part of a decrease in nutrient concentrations in chrysanthemum at increased CO_2 concentrations, was explained as a nutrient dilution of the leaf tissue due to starch accumulation by Kuehny *et al*. (1991). However, this dilution by starch, only account for some of the nutrients, and only for part of the variation between treatments. This relation was confirmed in the results of this thesis (Kjær *et al*., Paper I, Kjær *et al*., paper II)

Photosynthesis and carbohydrate metabolism

LNT have been shown to delay the gradual increase in photosynthesis during the early hours of the day in *Phaseolus vulgaris* (Izhar and Wallace, 1967), and in different pot plants (Rasmussen and Andersen, 1976), who also reported, that the photosynthetic rate did not reach the same level as in plants grown at an ambient night temperature. In chrysanthemum, LNT has been shown not to affect plant photosynthesis (Kohl and Thigpen, 1979). However, in the results of this thesis, low temperatures of 12ºC and 8ºC of the preceding night delayed the gradual increase in photosynthetic rate and also the maximum photosynthetic rate of *Chrysanthemum x morifolium* grown in a climate chamber, where the photosynthesis was measured from the light was turned on, and the following four hours at a temperature of 18ºC (Figure 2.1). An decrease in the approximated maximum photosynthesis at a night air temperature of 8ºC was linear related to an increase in starch content of the leaves (Kjær *et al*., Paper II), which suggested that an end-product limitation of photosynthesis occurred in the leaves. However, a higher N content in the shoots of the chrysanthemum plants grown at a night air temperature of 8ºC and with root zone heating, and an increase in the approximated maximum capacity of photosynthesis in this treatment, suggested that the photosynthetic capacity was increased in plants with root zone heating,

because there were more N-containing proteins available for carbon assimilation in the reductive pentose phosphate pathway, and more nitrogen in the thylakoids, which is known to be proportional related to the chlorophyll content of these organs (Evans, 1989).

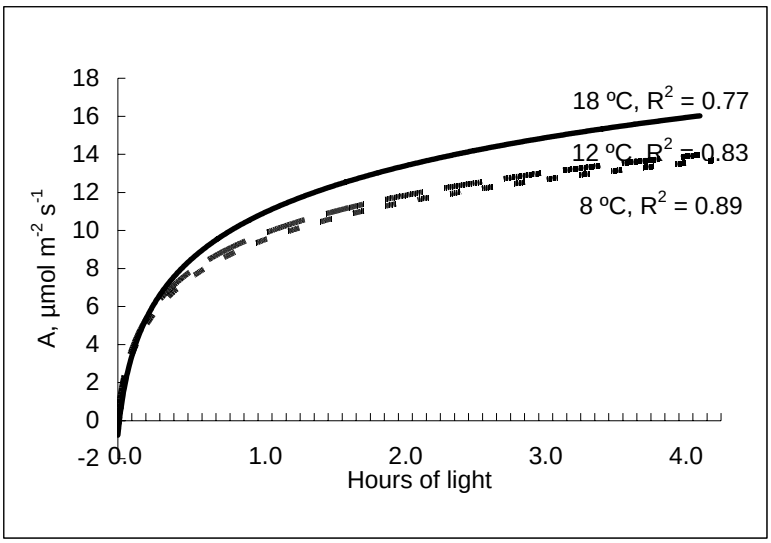

Figure 2.1
The figure show fitted curves of CO_2 assimilation rates (µmol m^{-2} s^{-1}) of *Chrysanthemum x morifolium* grown in a climate chamber at 18ºC day temperature, and three different temperatures (18ºC, 12ºC and 8ºC) during the night. The CO_2 assimilation was measured on three plants in each treatment from the onset of light, and the following four hours of the light period. The day temperature of 18ºC was reached after 15 min light in the 12ºC and 8ºC night temperature treatment, and the maximum irradiance of 430 µmol m^{-2} $s^{-1}$was reached after 1 h in all treatments. The CO_2 level in the chamber was approximately 570 µl l^{-1} throughout the measuring time in all treatments.

It is known that LNT can decrease the diurnal turnover of carbohydrates and increase starch accumulation in the leaves of cotton and arabidopsis (Warner *et al*., 1995; Strand *et al*., 1999), and in chrysanthemum (Kjær *et al*., paper I). However, it is important to note, that these studies were all performed under climate chamber conditions, and it is not known to date, how diurnal changes in carbohydrates are affected by conditions with fluctuating light, temperature, CO_2 and humidity. However, it is shown in the results of this thesis, that less starch is accumulated in the leaves at LNT under fluctuating light and temperature in a greenhouse (Kjær *et al*., Paper III), which suggest that the diurnal turnover of carbohydrates in a fluctuating climate, is not strictly related to starch accumulation during the day, and starch degradation during the night as suggested by Zeeman et al. (2007). In an experiment by Fondy *et al*. (1989) it was shown that starch accumulation in plants of sugar beat and bean started later and stopped earlier in a climate with a sinusoidal light regime, which simulates a natural light period where the light is gradually increased during the start of the light period, and gradually decreased during the end of the light period, than if the plants were grown in a climate with an abrupt change in the lights-on/lights-off regime.

Respiration

Plant respiration provides the driving force for biosynthesis, cellular maintenance and active transport in plants. Respiration couples the production of ATP, reducing equivalents and carbon skeletons to the release of 30-65% of the daily photosynthetic carbon gain in

plants as CO_2 (Atkin and Tjoelker, 2003). Respiratory losses consist of growth respiration, which is closely related to photosynthesis, and maintenance respiration, which is related to biomass accumulation. The respiratory process is temperature-sensitive, but much is still unknown about the dynamic response of respiration to short and long term changes in temperature. The short term response to lowering of the temperature is an abrupt decrease in respiration, whereas the long term response to low temperatures is more complex and involves acclimation, which may result in unchanged rates of respiration at low temperatures and an increase in respiration, when measured at higher temperatures (Atkin and Tjoelker, 2003).

The long term exposure of cold-sensitive cotton plants to night temperatures of 19ºC and 15ºC, resulted in a reduction of 42% in the respiration at 15ºC, in comparison with a night temperature of 28ºC, whereas the acclimated respiratory rates of plants grown at 19ºC during the night nearly equalled the rates of plants grown at the 28ºC night temperature (Lawrence and Holaday, 2000). Furthermore, Merrit *et al*., (1992) reported increased maintenance respiration of petunia and geranium at 21ºC, when the plants were grown at a night temperature of 7ºC in comparison with 15.5ºC. The authors suggested that any reduction in maintenance respiration during the night at LNT would result in a higher maintenance respiration during the day. From these results, it is suggested that the acclimation of respiration to LNT depend on the sensitivity of the plant species to low temperatures. The decrease in the photosynthetic rate of chrysanthemums grown at night temperatures of 12ºC and 8ºC in this thesis (Kjær *et al*., Paper I; Kjær *et al*., Paper II) may have resulted from an increased maintenance respiration during the day as shown in Merrit et al., (1992), which indicate that the respiratory rates during the night were not acclimated to the low temperatures; however, as respiration was not measured in the present studies, it is not possible to resolve this hypothesis.

2.3 Plant development

Plant size and flower development

Increased DM content of the shoot increased total leaf area at flowering in the chrysanthemum cultivar 'May Shoesmith' grown at split night temperatures, where the low temperature of 10ºC was applied for up to 11 h 30 min of the night during a short day treatment of 9 h day and 15 h night (Bonamino and Larson, 1980). The same trend was reported for some cultivars of chrysanthemum by Parups and Butler (1982); however, in that study, the specific leaf area ($cm^2\ mg^{-1}$, SLA) was unaffected at flowering. This indicated that the increase in total leaf area was mainly an effect of an increased number of leaves, or leaf size, in the LNT treatment, possibly as an effect of a longer flower initiation time (Van der Ploeg and Heuvelink, 2006). When plants of *Chrysanthemum x morifolium* were gown in a long day treatment, LNT decreased both leaf area and the number of leaves, in contrast to an increase in leaf DM (Kjær *et al*., paper I). All plants in the present experiment were harvested at the same time, illustrating that although no differences were found in total plant DM between the different treatments at a certain time, LNT had a significantly influence on the vegetative development of leaves.

Time to flowering is defined as the number of days between the start of the short day period, and flowering. The optimum temperature for flower formation in chrysanthemums lies between 17 and 22ºC and is cultivar-dependent. The short day period can be divided into a period of flower initiation, and a period of flower development, and both periods are sensitive to LNT; however, sensitivity decreases, as the plant matures (Van der Ploeg and Heuvelink, 2006). LNT in the short day period delays time to flowering in several studies on chrysanthemums (Kohl and Mor, 1981; Bonamino and Larson, 1980, Karlson and Heins, 1986), the trend is also seen in campanula (Niu *et al*., 2001), in roses (Van der Berg, 1987), and in several bedding plants (Merritt and Kohl, 1991). However, the delay is probably more related to the average temperature of day and night (AT), than to the average night temperature (ANT) (Van der Ploeg and Heuvelink, 2006). The number of flowers in chrysanthemum decrease, as an effect of LNT (Carvalho et *al*., 2005); however, the effect is cultivar-dependent (Parups and Butler, 1982). Although, the number of flowers decrease at LNT, the size of the individual flowers often increase (Willits and Bailey, 2000; Carvalho *et al*., 2005).

Plant height

One of the problems with growing plants at LNT and high day temperatures is an increased plant height due to a larger positive difference between day and night temperature (a positive DIF). If night temperatures are decreased in relation to day temperatures (a negative DIF), internode elongation and stem length will increase in many plant species. This is a fact for a range of floricultural crops studied, where final plant height is a matter of concern (Erwin *et al*., 1989; Myster and Moe, 1995), although tropical species have been shown to be less sensitive, due to their area of distribution. To understand the biological background of DIF, it is important to know how internode elongation responds to changes in day and night temperature. In chrysanthemum, the rate of stem elongation is greatest at the transition between night and day (Erwin and Heins, 1988; Myster and Moe, 1995). However, in *Campanulla isophylla,* stem elongation is high throughout day and night (Torre and Moe, 1998). When the night temperature is high, in comparison with day temperature, it is possible to shorten the period of stem elongation (Tutty *et al*., 1994, Torre and Moe, 1998). The mechanism is unknown, but interestingly Kaufmann *et al*. (2000) demonstrated, that root elongation was high during the night, and low during the day, in treatments with 27ºC night temperature and 19ºC day temperature, and the trend was opposite in treatments with night temperatures of 18ºC and day temperatures of 25ºC, The daily mean temperature was 22ºC. They suggested, that mobilization of available assimilates to rapidly growing roots during the night, when the temperature was high, decreased the supply of carbohydrates available for stem elongation, which thereby restricted the length of the growth period. In the results of this thesis it was shown that the export of carbohydrates from the leaves was inhibited at night temperatures below 12ºC (Kjær *et al*., Paper I), which might explain why the positive DIF in the treatments with LNT did not increase stem length of the chrysanthemums in the present studies.

2.4 Conclusions

It can be concluded that the climatic conditions, during the day, including sunlight, CO_2 concentrations and temperature, determines the amount of carbohydrates available to the plants. In addition, the temperature during the night, determines in which way the plants allocate and use the carbohydrates. Therefore, it seems clear that lowering the night temperature within a certain range does not decrease plant DM production. However, lowering the temperature may decrease maintenance respiration, which may be responsible for the delay in flowering and increased production time of the plants. Although, the effects of LNT are mainly negative, different species and cultivars respond to different degrees, and cultivars may even be bread to cope with lower temperatures (van der Ploeg *et al*., 2007). Furthermore, a delay in production time may be acceptable, if the plants have larger flowers, and if the energy cost of the production is lower. Also, the effects of LNT may disappear when plants are grown in a dynamic climate. In the dynamic climate high day temperatures are often followed by a slow gradual decrease in night temperature, whereas low day temperatures are followed by low night temperatures, which is in contrast to a climate, controlled according to the average day temperature (AT).

3. Carbohydrate metabolism and nitrate uptake and assimilation

3.1 Introduction

It is clear from chapter two, that growth and development of floricultural crops in response to LNT is well-studied. Less is known about how the physiological responses are affected, when lowering the temperature. Many of these processes are temperature-dependent, and expected to show a response to low temperature. However, when the temperature is only low during the night, less is known about which processes are the most limiting for plant growth, and whether plants are able to compensate for these limitations by optimising the processes during the day. In this chapter, the current knowledge about carbohydrate metabolism and NO_3^- uptake and assimilation is reviewed. Both processes are known to show a diurnal pattern. Starch accumulation occurs in the leaves during the day, possibly as an overflow, because carbohydrate assimilation exceeds the current sink demand. The accumulated starch is degraded during the night as a supply of sucrose, when photosynthesis is not present. Nutrient uptake also show a diurnal pattern which vary considerably between different nutrients. The focus in this thesis is on NO_3^- uptake and transport, which show a distinct pattern related to carbohydrate metabolism during night and day.

3.2 Carbohydrate metabolism

The occurrence and function of starch in leaves

Starch is an important carbohydrate reserve in many plants. Two types of starch are accumulating in plants distinguished according to function: Reserve starch accumulates inside the amyloplast in storage organs, such as tubers and seeds; transitory starch is synthesized directly from photosynthetically fixed carbon dioxide inside the chloroplasts, and serves as a short or medium term carbohydrate reserve for periods of darkness, and when photosynthesis is low. The extent, to which starch accumulates in leaves, and the magnitude of the diurnal changes in starch content, vary considerably among species. In *Arabidopsis thaliana*, starch is the major carbohydrate accumulated. When grown for a 12 h photoperiod, starch is synthesized throughout the photoperiod and almost entirely degraded during the dark period (Lin *et al*., 1988). Similar results have been obtained with pea, spinach and korean ginseng, (Stitt *et al*., 1978, Gerhardt *et al*., 1987 Miskell *et al*. 2002).

Some plant species only accumulate little starch. Instead, sucrose or fructans, which are polymers of fructose, are stored in vacuoles of the mesophyll cells. Starch metabolisms of these plants have only received sparse attention, and little is known about the physiology and regulation. One study performed on *Lolium temulentum*, showed that starch accumulated in mesophyll cells, simultaneously with the accumulation of sucrose. However, the accumulation of starch ceased after 12 h reaching an amount of only 0.6% of the fresh weight, whereas the sucrose accumulation continued (Cairns *et al*., 2002).

There is more than one opinion about the function of starch in plant leaves, but two models dominate. In the overflow model, starch synthesis is considered to occur primarily as an overflow for newly assimilated carbon, when assimilation exceeds the demand for sucrose (Stitt and Quick, 1989). In support of this model, intact leaves of sugar beat and pea, has been shown to have a low rate of starch synthesis, in relation to sucrose synthesis, during the early part of the day, whereas the starch synthesis increased, in accordance with a rise in the sucrose content of the leaf, later during the day (Stitt *et al*., 1978; Fondy and Geiger, 1982). Further support for the idea, is given by the general observation that restriction of carbon export from the leaves leads to an increased rate of starch synthesis. For example, a transgenic potato plant with either reduced activity of the triose phosphate transporter (TPT), which is responsible for transport across the membrane of the chloroplast, or a sucrose-proton translocator, which is involved in phloem loading, had increased starch synthesis, in accordance with decreased capacity for carbon export (Heineke *et al*., 1994). The second model of starch metabolism, proposes that starch may provide a source of carbon for maintenance during the following night (Trethewey and Smith, 2000). In sugar beat an endogenous circadian rythm, was shown to ensure that the amount of starch synthesized during the day, was equal to the demand of carbon during the following night (Li *et al*., 1992). This view of starch metabolism being regulated by a circadian rythm, was recently supported by Lu *et al*. (2005). Patterns of starch mobilezation in arabidopsis, were compared between plants grown in long days, versus plants grown in short days, and plants shifted from short days to long days, and vice versa (Figure 3.1). A larger build up of starch, followed by a faster starch breakdown rate in long days was reported, which suggested that plants may, in some way, sense day length and adjust their rate of starch degradation during the dark period. In accordance with this, Zeeman *et al.* (1999) reported a relatively constant starch degradation rate throughout the night in arabidopsis. The phenomenon is remarkable, and suggests that the starch degradation rate is somehow controlled by the amount of starch available, and the expected length of the dark period.

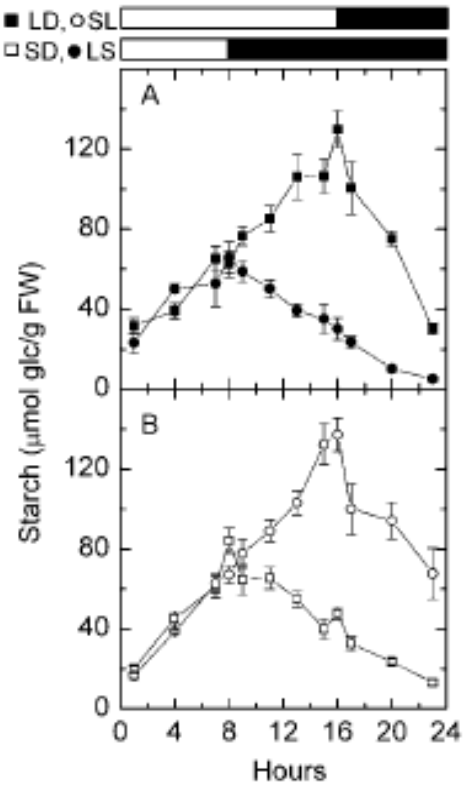

Figure 3.1
Diurnal changes of starch in long day (LD), long-to-short day (LS), short day (SD), and short-to-long day (SL). A, LD (black squares) and LS (black circles). B, SD (white squares) and SL (white circles). White bars and black bars on the top indicate days and nights, respectively. Values are mean of 6, SE (n=5). FW, fresh weight. Figure taken from Lu *et al*. (2005).

Both models of the role of leaf starch seems likely, and it is proposed that starch is synthesized both as an overflow for newly assimilated carbon, and as a source of carbon during the night (Trethewey and Smith, 2000). However, the relative importance of the two roles is highly dependent on plant species, and growth conditions. An overview of starch biosynthesis, starch structure and starch degradation in the leaves of plants was recently given by Zeeman *et al*. (2007). However, as most studies reviewed were performed under climate chamber conditions, their conclusions does not provide us with an understanding of how starch biosynthesis and degradation is regulated in an environment with fluctuating light and temperatures, which is the natural environment of plants. Results in this thesis indicate, that there might be differences in starch metabolism of plants grown in climate chambers and greenhouses with fluctuating light and temperatures (Kjær et al., Paper I; Kjær *et al*., Paper III).

The biosynthesis and degradation of starch

The pathways of starch biosynthesis and degradation in plants are still not fully understood; although, much research has been performed in this area in recent years. Most research has been performed on arabidopsis, and species-dependent variations in the pathways are unknown. Knowledge about the pathway of starch biosynthesis and degradation is important, if we are to understand, how circadian rhythms and climatic parameters contribute to the regulation between starch and sucrose biosynthesis. Furthermore, it is important to know whether plants are able to degrade starch, which has been accumulated as an effect of low temperatures or increased CO_2 concentrations. This will supply us with knowledge about the ability of the plants to store resources temporally, and use them later when needed.

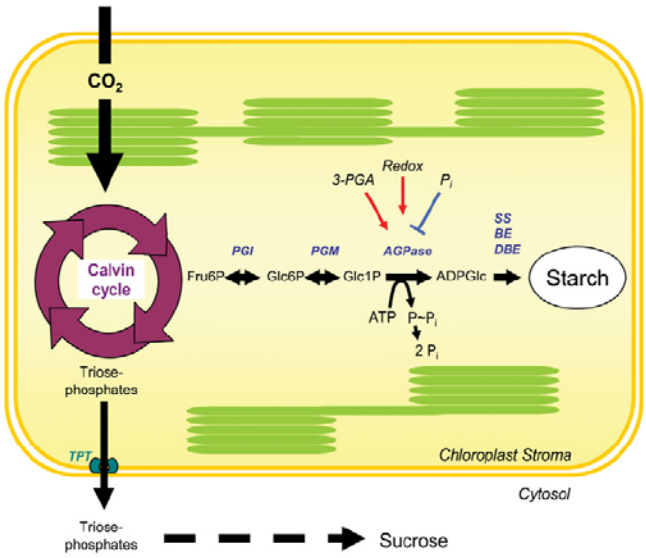

Figure 3.2
Pathway of starch synthesis in chloroplasts. Carbon assimilated via the calvin cycle, also known as the reductive pentose phosphate pathway, is partitioned with a fraction exported to the cytosol for sucrose synthesis via the triose phosphate/phosphate translocator (TPT) and a fraction retained in the chloroplast for starch synthesis. A high $3PGA/P_i$ ratio stimulates the AGPase, and the redox activation of AGPase is probably medieated by the chloroplast ferredoxin/thioredoxin system which controls activation/inactivation of AGPase during day/night. Abreviations: Fru6P, Fructose 6-phosphate; Glc1P, glucose 1-phosphate. Figure taken from Zeeman *et al*. (2007).

The starch biosynthetic pathway has generally been considered to take place exclusively in the chloroplast, and to be segregated from the sucrose biosynthetic pathway, that takes

place in the cytosol (Figure 3.2) This view is supported by a range of mutant plants with reduced, or undetectable activities of plastidial PGM or AGPase, which are unable or only have restricted capacity to synthesise starch (Caspar *et al*., 1986; Lin *et al*., 1988; Trethewey and Smith, 2000). Direction of newly fixed carbon into starch synthesis is achieved by phosphate dependent signals (Trethewey and Smith, 2000). The classical view, supporting the overflow model, argues that when photosynthesis is high, relative to the demand of sucrose, then the ratio of 3-phosphoglycerate (3-PGA) to Pi should be high, and this would activate AGPases and generate a high flux through the starch biosynthetic pathway (Figure 3.2).

Starch degradation in arabidopsis leaves was recently reviewed by Smith *et al*. (2005), and a model for the suggested pathways is shown in Figure 3.3. Uncertainty about a large part of the degradation pathways still exists, and the mechanisms that control starch degradation are not yet understood. More knowledge in this area may provide us with a better understanding of how plants sense daylength, temperature and other climatic parameters and adjust their rate of starch degradation to the preceding photoperiod.

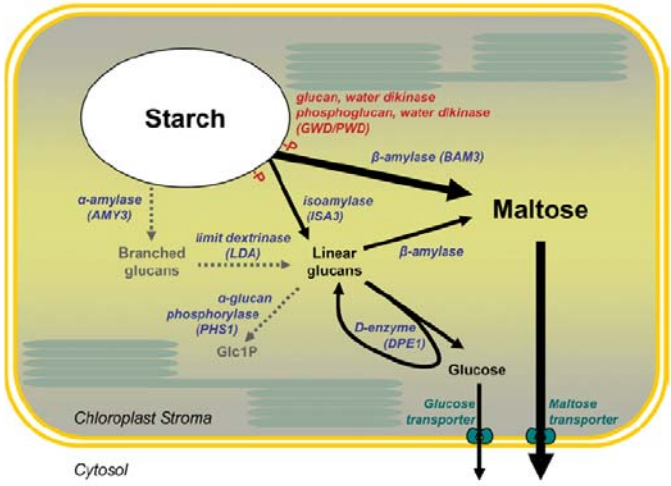

Figure 3.3
A model for the suggested pathways of starch degradation in arabidopsis chloroplasts. Starch is hydrolysed to maltose and glucose during the dark, but the importance of the glucose transporter for starch breakdown has not yet been established. The thickness of the arrows reflects estimates of respective fluxes. Figure taken from Zeeman *et al.* (2007).

Accumulation of starch in response to environmental stresses

Starch content of plant leaves responds to various types of environmental stress. Low nitrogen in the growth medium have been shown to increase the starch content of chrysanthemums and pelargoniums (Druege *et al*. 2000; Druege *et al*., 2004), increased CO_2 concentration have been shown to increase starch accumulation in some chrysanthemum cultivars (Kuehny *et al*., 1991; Kjær et al., Paper III), in tobacco (Geiger *et al*., 1999) and in potato (Katny *et al*., 2005). Water stress has been shown to increase starch accumulation in older leaves of arabidopsis, which were adapted to mild water stress (Lu and Sharkey, 2006) and low temperatures have been shown to increase the accumulation of leaf starch in arabidopsis, tomato and chrysanthemums (Venema *et al*., 1999; Strand *et al*., 1999; Kjær *et al*., Paper I). In arabidopsis, the diurnal turnover of leaf carbohydrates were significantly damped, when plants were shifted from 23ºC to 5ºC in the dark period, and after 10 days leaves contained relatively large stable pools of starch and soluble carbohydrates. The mechanism proposed, is a chilling-induced inhibition of phloem-export, leading to accumulation of carbohydrates in the leaves (Gamalei *et al*., 1995), which then

repress photosynthetic gene expression (Strand *et al*. 1997). Another mechanism operating at low temperatures, is an increased production of soluble sugars. Recently a review was published by Kaplan *et al*. (2006), which concluded that β-amylases have a significant role in low temperature stress tolerance, by increasing maltose and other soluble sugars that can act as cryoprotectants (Yano *et al*., 2005).

3.3 Nitrogen (N) uptake

The NO_3^- uptake mechanism

Nitrogen (N) is the nutrient of highest concentration in higher plants and represents 2 – 5% of total plant dry weight. Plants require N throughout their development, and N is a component of proteins, nucleic acids, coenzymes and secondary metabolites. The main N-sources are nitrate (NO_3^-) and ammonium (NH_4^+); however, NO_3^- is the most commonly used compound by many plants, and in the focus of this chapter. NO_3^- is taken up by the roots, and either reduced, stored in vacuoles, or transported to the shoots for reduction and storage in the vacuoles. There is a close connection between NO_3^- assimilation and carbohydrate metabolism, which will be discussed later.

Most NO_3^- uptake takes place just behind root meristem (Taylor and Bloom, 1998); however, studies on N transporter genes, suggests that the mature part of the root system are also significant sites of NO_3^- uptake (Nazoa *et al*., 2003). NO_3^- is actively transported across the plasma membrane of epidermal and cortical cells of roots, and the transport process requires energy (Figure 3.4). It is generally accepted, that the uptake of NO_3^- is coupled with the co-transport of 2 H^+, and therefore uptake of NO_3^- depend on ATP supply to the H^+ ATPase, that maintains the H^+ gradient across the plasma membrane (Miller and Cramer, 2004).

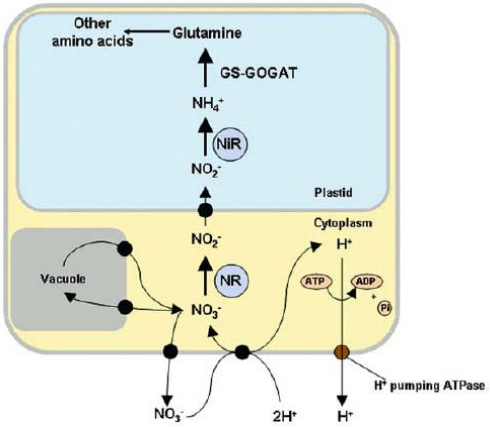

Figure 3.4
Schematic diagram of NO_3^- uptake and assimilation by plant cells. The NO_3^- is actively transported across the plasma membrane coupled to the cotransport of 2 H^+, then reduced to NO_2^- via Nitrate Reductase (NR) and further to NH_4^+ and amino acids via Nitrite Reductase (NiR) and the gluamine synthetase/glutamate-2-oxoglutarate aminotransferase (GS/GOGAT) pathway. Taken from Miller and Cramer (2004).

The N Transport

NO_3^- is either assimilated to amino acids in the roots, and transported to other plant organs via the xylem, or transported directly as NO_3^- in the xylem. Whether plants do either, or both, is species-dependent, but also dependent on climatic factors. The xylem sap concentrations of NO_3^- have been shown to be diurnally regulated, and related to changes in the rate of transpiration (Rufty *et al*., 1984; Siebrecht *et al*., 2003). Amino-N is

transported within the plant, through both the xylem and phloem, and the unloading/loading of amino-N in these two transport systems results in an extensive N-cycling between shoot and root, which may involve a large amount of the total N in the plants. It is believed that this combined pool of N in shoot and root, is involved in the regulation of N uptake (Cooper and Clarkson, 1989). The major amino acid components in xylem and phloem are the amides, glutamine and asparagine, and the acidic amino acids, glutamate and aspartate.

The N uptake and transport in response to low temperature

When plants of *Zea maize* are grown at different temperatures in the root zone, the uptake and transport of N to the shoots depend more on the shoot demand for N (the temperature of the shoot), than on the direct temperature effects on the root system (Engels *et al*., 1992). This relation was confirmed by Castle *et al*. (2006), who showed, that when plants of *Trifolium repens* were grown at a temperature of 8ºC in both root zone and air, N uptake was not limited; however, the N was preferentially stored in the roots, due to a limitation in the movement of N from roots to shoots. These results suggest that the N uptake mechanism is not directly limited by low temperature. Further indications, that the capacity of NO_3^- uptake is regulated by plant N demand, have been obtained by imposing N-deficiency. Plants that were N-starved for periods of hours to days, developed an enhanced capacity to absorb NO_3^-, when the ion was re-supplied (Touraine *et al*., 2001). Furthermore, studies were the root system of intact plants is divided in two compartments, containing either an N-free solution, or an N-containing solution, have demonstrated that the NO_3^- uptake capacity is regulated by shoot signals (Lainé *et al*., 1995). Another factor, which influence the ability of plants to cope with changes in soil temperature, or N availability, is the ability of the plants to change the root: shoot ratio. An increased root absorbing surface, relative to shoot size, will decrease NO_3^- uptake per unit root, and vice versa (Clarkson *et al*., 1988). The observations show that changes in the uptake capacity of NO_3^-, in response to environmental stresses, always should be viewed in conjunction with the actual demand of the plants for N (BassiriRad, 2000). In the results of this thesis, it is indicated, that when the temperature is only low during the night, plants may compensate for a lower NO_3^- uptake capacity during the night, by increasing the NO_3^- uptake capacity during the day (Kjær et al., Paper I). It confirms that the N uptake system of plants, is highly adaptive to changes in the environment.

The effect of low temperature on N transport from root to shoot, is thought mainly to occur as an effect of decreased xylem transport, due to a decrease in the hydraulic conductance (Castle *et al*., 2006). In conjunction with this, a lower rate of xylem transport in response to a decrease in transpiration, and stomatal closure, explain the decrease in NO_3^- transport to the shoot during the night (Rufty *et al*., 1984) and in the results of this thesis (Kjær *et al*., Paper I).

3.4 The relation between nitrogen assimilation and carbohydrate metabolism

Photosynthetic N assimilation

The regulation of N assimilation in leaves and roots are extensively studied and reviewed (Meyer and Stitt, 2001; Miller and Cramer, 2004). Most information is available for NO_3^- assimilation and its regulation. The substrate NO_3^- is the primary regulatory signal; although, other signals such as light, carbon metabolism, pH regulation inside the cell, circadian rythms, and the ion and assimilate flow at the cell and whole plant level, also influence the regulation of NO_3^- assimilation (Miller and Cramer, 2004). The interactions between the different aspects of NO_3^- assimilation are complex, and only some interactions are outlined in this section.

In the leaves, NO_3^- is reduced to NO_2^- via nitrate reductase (NR) (Figure 3.4). The capacity for nitrate reduction (NR activity) increases with light, NO_3^- availability, and sugar availability, and decrease in response to low CO_2 concentrations (Foyer *et al*., 2001). NO_2^-, arising from the NR-action, is transported into the chloroplasts, where subsequent reduction to NH_4^+ occur, catalysed by nitrite reductase (NiR). Reduced ferredoxin (Fd) from the light reaction of photosynthesis is the reductant of NiR. The product of the second reaction, NH_4^+ is readily converted to amino acids via the GS/GOGAT cycle (Meyer and Stitt, 2001).

Diurnal changes in NO_3^- assimilation

The mRNA levels coding for the NR protein show diurnal variation, the level of mRNA increase during the night to a maximum in the early morning (Miller and Cramer, 2004). Illumination stimulates translation of the mRNA, and inhibits degradation of the NR protein. Illumination also stimulates activation of NR, and high rates of nitrate assimilation are achieved during the first part of the light period. The NO_3^- assimilation rate highly exceeds the rate of NO_3^- uptake, and the rate of flux through the GS/GOGAT pathway, which causes the NO_3^- pool to be depleted, and N to be accumulated as intermediate products, such as ammonium and glutamine. During the second part of the light period, and during the night, NR activity and NO_3^- assimilation are inhibited, which allow the GS/GOGAT pathway to assimilate the intermediate products further to amino acids. The NR activity is regulated by end-products of N assimilation, and carbohydrate products from photosynthesis. Glutamate is believed to play a major role, but also malate and low levels of sugars lead to a marked decrease in NR activity. In plants growing under conditions with low light, low levels of sugars lead to a decrease in NR activity, possibly as an effect of inhibition of the GS/GOGAT pathway. The result is a situation where plants become C and N limited (Stitt *et al*., 2002).

Co-ordinated regulation of carbon metabolism and nitrate assimilation

The NO_3^- assimilation is closely connected to primary carbon metabolism (Figure 3.5). Products of NO_3^- assimilation are invested in proteins and chlorophyll of the photosynthetic apparatus. the photosynthetic capacity of leaves is related to the N-content, and there is a linear relation between N and ribulose 1,5 biphosphate (Rubisco), which is the primary acceptor of CO_2 in the reductive pentose phosphate pathway (Evans, 1989). Furthermore, NO_3^- assimilation requires a continuous supply of energy and carbon skeletons. Therefore, photosynthetic products are partitioned between carbohydrate synthesis, and the synthesis of amino acids. The partitioning is flexible, and varies between plant species, the developmental stage of the plant, and environmental conditions.

During NO_3^- assimilation, carbohydrate synthesis is decreased, and more carbon is converted via glycolysis to phosphoenolpyruvate carboxylase (PEPC). The PEPC has two functions during NO_3^- assimilation, it provides malate for pH regulation of the cell, and it provides 2-oxoglutarate (2-OG), which is the primary carbon acceptor for ammonium (Foyer *et al*., 2001). The result is a shift in the priorities for carbon use during the diurnal cycle. During the first part of the light period, when NO_3^- assimilation is high, carbon metabolism is directed into malate synthesis, which ensures pH regulation in the leaves during NO_3^- assimilation. Later during the day, when NO_3^- assimilation decreases, carbon is directed into production of 2-OG in order to facilitate the production of amino acids.

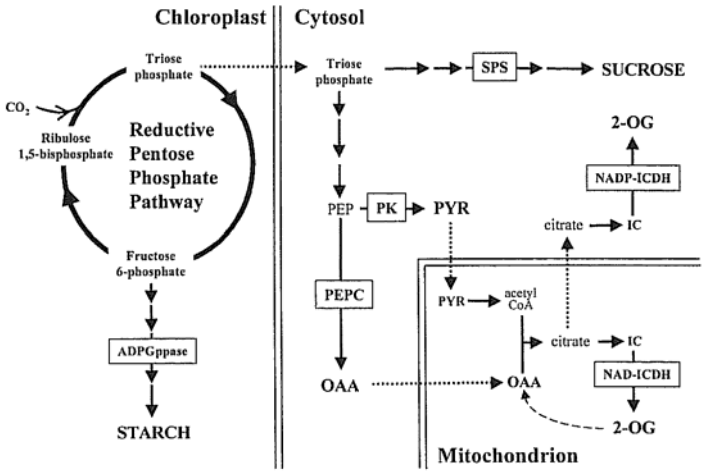

Figure 3.5
Carbon partitioning between carbohydrate synthesis and respiratory generation of 2-oxoglutarate (2-OG), and other organic acids (OAA, PYR). Abbreviations: ADPGppase, ADP-glucose pyrophosphorylase; IC(DH), isocitrate (dehydrogenase); OAA, oxaloacetate; PEPC, phosphorenolpyruvate carboxylase; PK, pyruvate kinase; PYR, pyruvate; SPS, sucrose phosphate synthase. Figure taken from Foyer *et al*. (2001).

The C/N balance and source/sink relations in plants

The relation between carbohydrate metabolism and NO_3^- assimilation, and the response of plants to C and N-status, highlights the plasticity of plant development. In a study by Geiger et al. (1999), it was shown, that when plants were grown under conditions where the availability of N is low, or the CO_2 concentration is high, excess carbohydrates may accumulate in the leaves as starch. Accumulation of starch may function as a sink for C, that may help the plants to adjust the C/N balance. Geiger et al. (1999) also showed that low N availability at high CO_2 concentrations decrease photosynthetic capacity and the level of sugars in the leaves, possibly as an effect of the linear relation between rubisco and N

content of the leaves (Evans, 1989). When plants are grown at low night temperatures, which are the topic of this thesis, an altered balance between source and sink activity also leads to accumulation of starch in the leaves, a decrease in photosynthesis, accompanied by a lower N-concentration in the plants (Kjær *et al.*, Paper I, Kjær *et al*., Paper II). These results support, that the relationship between N assimilation and carbon metabolism is in a complex pattern, and it is suggested, that the down-regulation of photosynthesis in plants grown at low night temperatures, depend more on the C and N-status of the leaves than on the carbohydrate status alone. This relation was also suggested by Paul and Driscoll (1997).

4. Methods and experimental work

4.1 Outline of experimental work

Three experiments were performed to show how nutrient uptake and transport, and transport of carbohydrates in plants are affected by the temperature during the night. The plant chosen for the experiments was *Chrysanthemum x morifolium* L. cv. 'Coral Charm'. In the first two experiments (Paper I and II), plants were grown in water-based nutrient solution cultures, in climate chamber. This was done to eliminate natural fluctuations in the light and temperature. Water was used instead of peat, to eliminate possible effects of temperature on nutrient transport in a heterogeneous growth substrate.

In the last experiment (Paper III), plants were grown in peat and in a greenhouse, with the purpose of extrapolating the results from the controlled environments in the climatic chambers to a production site, where plants were also subjected to the influence of reduced night temperature in combination with external factors, such as nutrient transport in peat and humidity of the air. Plants were grown in long day conditions in all three experiments, to ensure that plants remained vegetative, as chrysanthemums flower at short day conditions with a photoperiod of less than 12 h.

Experiment 1 (Paper I)

The aim of this experiment was to find the primary limiting factors, which may limit plant growth at low night temperature, by studying plant physiological responses. The experiment included plant growth analysis, including a morphological analysis of root growth, and an overnight study of NO_3^- uptake and carbohydrate distribution in the plants. The experiment was replicated twice, due to problems with maintaining the planned temperature regime in one climate chamber in the first experiment.

The results demonstrated that reduced night temperatures down to 8ºC did not affect overall dry matter (DM) production, possibly as an effect of reduced respiration and an increase of starch in the leaves, which result in less carbohydrate available for formation of new leaves, leaf expansion and root DM production. Daily NO_3^- uptake of the chrysanthemums was not affected; however, a night temperature of 8ºC decreased the NO_3^- uptake rate during the night, which suggested that the plants compensated by having similar or slightly increased NO_3^- uptake rates during the following day. The increased starch content of the leaves in plants grown below 12ºC during the night, was an effect of a limitation in the export of carbohydrates from the leaves, and probably the most limiting factor to the chrysanthemums grown at LNT. It indicates that there was an imbalance in the plant energy absorption in the form of carbohydrates from photosynthesis, and the use of carbohydrates for maintenance during the night,

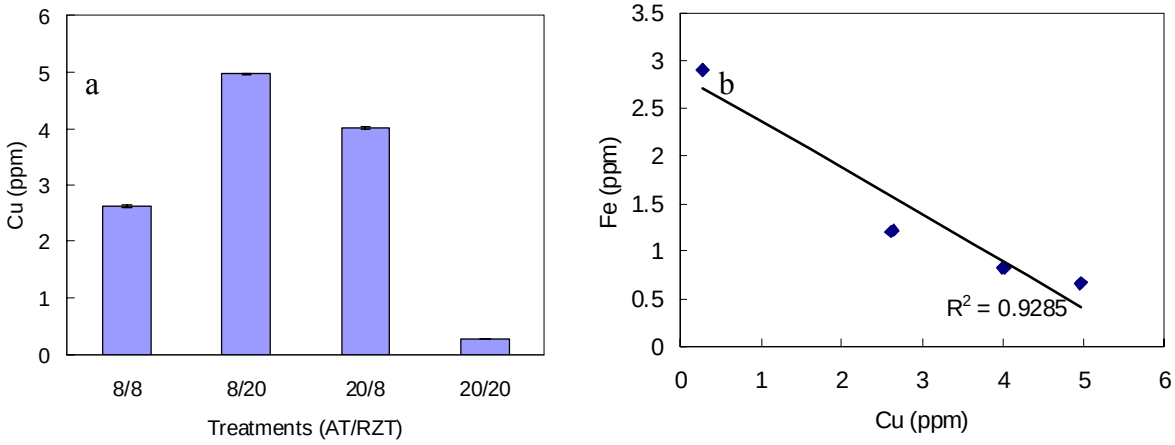

Fig. 4.1
Copper (Cu) delivery from copper coated temperature sensors in three thermal water baths. The temperature sensor of the fourth thermal water bath was not coated with Cu. (A) Cu concentration of nutrient solution in four treatments with four combinations of night air temperature and night root zone temperature (NAT/NRT). (B) The relations between Iron (Fe) concentration and Cu concentration of the nutrient solution.

Experiment 2 (Paper II)

The aim of this experiment was to study, whether the negative effects of LNT on plant growth and physiology of *Chrysanthemum x morifolium*, could be overcome by heating the root zone of the plants. The experiment was replicated three times, due to problems with a temperature sensor placed in the thermal water bath, which released unwanted ions of copper (Cu) to the nutrient solution in the first experiment (Figure 4.1a) The increase in Cu was linearly related to a decrease in iron (Fe) availability of the nutrient solution (Figure 4.1b). No explanation of this phenomenon was found; however, it may have occurred, because the ion of Fe changed place with the ion of Cu in the covering of the temperature sensor. Yellow colouring of plant leaves, indicated that the plants were suffering from Fe

Figure 4.2
Root rot in *Chrysanthemum x morifolium* grown at a day temperature of 20ºC and four combinations of night air and night root zone temperature (see figure). Root rot was thought to occur because of contamination of the nutrient solution culture system, most probably by *Fusarium oxysporum* from a different culture of chrysanthemum, which were rooted in soil and transferred to the nutrient solution culture in a test experiment.

deficiency or Cu toxicity. Chrysanthemums exposed to 5 µl l^{-1} Cu^{+} have been shown to have significantly reduced plant dry weight (Zheng *et al*., 2004).

In the second experiment the temperature sensor was covered with a plastic sheet; however, in this experiment, plants were attacked by root rot, due to a contamination of the nutrient solution culture system, and the majority of the plants had to be discarded (Figure 4.2). In the third experiment, no major problems occurred, and results are reported.

The results of the present paper demonstrate that root zone heating did not decrease starch accumulation at low night air temperatures, which rejected the hypothesis that increased root growth and activity of heated roots may provide a larger sink for carbohydrates and increase the carbohydrate export from the leaves and decrease the leaf starch content. Furthermore, a close negative relation between approximated CO_2 assimilation capacity and the starch content of the leaves was found, which confirmed results from the literature on an end-product limitation of accumulated carbohydrates on photosynthesis.

The daily NO_3^- uptake was not affected, and the NO_3^- uptake during the night was not significantly increased by root zone heating, which rejected the hypothesis that increased temperatures increase NO_3^- uptake. Instead, the NO_3^- uptake is suggested to depend mainly on shoot demands, which confirm results from the literature (Lainé *et al*., 1993). The shoot N concentration decreased in response to low air, and low root zone temperature; however, the shoot N content only decreased in response to low root zone temperature, and it is suggested that the decrease in approximated maximum CO_2 assimilation at low root zone temperatures was related to the N-content of the shoot, as a relation between N content and photosynthetic capacity has been shown by Evans (1989).

Experiment 3 (Paper III)

This experiment aimed to study the results obtained in the former climate chambers studies when plants of *Chrysanthemum x morifolium* were grown under greenhouse conditions with fluctuating light and temperature. The experiment was performed twice, in the autumn of 2006 and again in the following winter of 2007, each time using three replicates. In contrast to the climate chamber experiments, plants were grown in peat.

A night temperature set point of 12ºC increased starch accumulation in the leaves, but not as much, as in the climate chamber experiments, and the low night temperatures did not have major influences on plant morphology and plant growth. A high [CO_2] set point of 900 µl l^{-1} was shown to have a contributing effect on the starch accumulation at LNT. However, there was a difference in the effect of temperature and [CO_2] between the autumn and winter experiment due to the unusually high outdoor temperatures during the autumn, which made it impossible to cool down to 12ºC during most of the nights. Furthermore, the high outdoor temperature during the autumn also resulted in lower CO_2 concentration in the high [CO_2] treatment due to a longer time of ventilation to obtain required day temperature.

4.2 Materials and methods

Plant material

The plant chosen for the work described in this thesis was *Chrysanthemum x morifolium* Ramat. cv. 'Coral Charm'. The genera *Chrysanthemum* comprises over 150 species and originated from China, where the cultivation of the plant started more than 1400 years ago. They are member of the Asteraceae, and modern cultivated chrysanthemums are complex hybrids involving *C. indicum* and *C. morifolium* **(**Manrique, 1993). Chrysanthemum is typically a short day plant, and the change from vegetative to generative growth takes place, when the photoperiod is shorter than 12 hours, whereas plants stay vegetative under long day conditions (> 14 hour light). Their optimal temperature range for growth is 18º - 20ºC (Van der Ploeg and Heuvelink, 2006)

Growth system

The growth system chosen for the work, was a nutrient solution culture system, where plants were grown in rectangular plastic containers with 8 l of nutrient solution, each containing three plants. Plant stems were placed in a slit of a circular styrofoam sheet, which was fastened in a plastic tube with a diameter of 9 cm, and a length of 18 cm. The tubes were fastened in the container lit, and when closing the container, the tubes were submerged in the container down to 1 cm above the bottom. This allowed full availability of the nutrient solution to all roots in the containers, and avoided tangling of roots from different plants (Figure 4.3). The decision to grow the plants in nutrient solution culture, instead of peat, was chosen on the basis of a preliminary experiment, which aimed to find the best suitable growth system for root morphology studies at different root zone temperatures. The main conclusion of this study, was that the nutrient solution culture system allowed easy examination of the root system, and easy harvest of root material, with the aim of studying diurnal changes in carbohydrate composition of roots. Furthermore, the system allowed an easy method in supplying $Na^{15}NO_3^-$ to the medium, as it was easy to replace the nutrient solution of the containers with a new nutrient solution containing a higher atom% of ^{15}N.

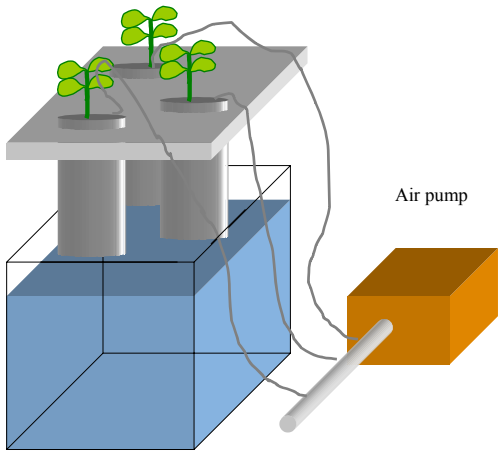

Figure 4.3
Model of the nutrient solution culture system used in the experiments of this thesis. The rectangular plastic containers were made of grey plastic, which allowed no light transmission. The plastic tubes were fastened to the lit, which allowed easy and non-disturbing lifting of the plants when pH, EC and water status was checked.

Carbohydrate analysis

The diurnal and long term changes in carbohydrate levels in plant leaves and roots were studied, in order to increase the knowledge on the ability of the plants to transport carbohydrates during the night and day. Plant material, harvested for carbohydrate analysis was quickly frozen in liquid nitrogen, in order to stop the enzymatic degradation of sugars, which may occur after the material is harvested. The carbohydrate analysis was carried out by the University of Copenhagen, Faculty of life sciences, Institute of Agricultural Sciences. Further information about the method is found in the material and methods part of Paper I.

The ^{15}N analysis

An overnight study of the uptake of the nitrogen isotope $^{15}NO_3^-$ was carried out, in order to study the effect of LNT on NO_3^- uptake. The ^{15}N analysis was carried out at a commercial laboratorium (Iso-Analytical ltd., United Kingdom). Further information on the method can be found on the website of the laboratory (*http://www.iso-analytical.com*):

Technique: EA-IRMS (elemental analysis - isotope ratio mass spectrometry).

For determination of ^{15}N and ^{13}C, the bulk material must first be converted to pure N_2 and CO_2 for analysis by the IRMS. In this technique, samples are placed in clean metal capsules and loaded into an automatic sampler. They are then dropped into a furnace held at 1000ºC where they are combusted in the presence of added oxygen. The metal capsules are flash combusted, raising their temperature in the region of the sample to ~ 1700ºC. The combusted gases are then swept in a helium stream over a combustion catalyst (Cr_2O_3), CuO wires (to oxidize hydrocarbons), and silver wool to remove sulphur and halides. The remaining gases, N_2, NO_x, H_2O, O_2, and CO_2 are then swept through a reduction stage of pure copper wires held at 600ºC. This step will remove any oxygen and convert NO_x to N_2. Water is removed by a magnesium perchlorate while CO_2 can be removed via a selectable Carbosorb™ trap. Nitrogen and carbon dioxide are separated by packed column gas chromatograph held at an isothermal temperature. The resultant chromatographic peak enters the ion source of the IRMS where it is ionised and accelerated. Gas species of different mass are separated in a magnetic field then simultaneously measured on a Faraday cup universal collector array. For N_2, masses 28, 29, and 30 are collected.

The pH and plant activity

The pH of the nutrient solution culture system was measured and corrected to 5.8 with NaOH and HNO_3 every day throughout the experimental period. The pH of the nutrient solution cultures fluctuated daily during the experiments. In the beginning, it mainly increased by 0.5 to 1 pH units. When NO_3^- is the major form of N supplied, plants absorb an excess of anions and there is a net efflux of HCO_3^- and OH^- resulting in an increase in pH. At the end of the experiment, pH decreased daily by 1.5 to 2 pH units, possibly due to

increased root activity and a larger root system as increased root respiration decrease pH. A better stability of the pH in the nutrient solutions may have been achieved, by increased periodic replacement of nutrient solution, a larger solution volume, or by buffering with soluble ligands or NH_4^+ (Parker and Norvell, 1999).

4.3 Conclusion

It was demonstrated in this thesis, that the nutrient solution culture system can be used in the study of plant physiological reactions to LNT. However, it turned out to be challenging to maintain pH in the system, and this may have interfered with the temperature treatments and caused differences in plant responses, which could not be detected directly. An increased awareness on pH regulation of the nutrient solution, must be included in future studies, when using the nutrient solution culture systems. Another limiting factor was the size of the system. Only a limited number of plants could be kept in the system at the same time, and when problems occurred to some plants, in for example, the second experiment (Paper II), all plants had to be discarded, because the nutrient solution was cycled through all the containers of a treatment. However, the easy harvest of root material was important in studying diurnal changes in carbohydrate levels and NO_3^- uptake into the root, and the root morphology studies were easily performed on root samples. However, differences between treatments were limited, which suggests, that although the method was good, the importance of the results was low, in comparison with the difficulties in growing plants in nutrient solutions instead of peat. Furthermore, it is important to note, that although root studies are easy done on plant roots, when grown in nutrient solution, the results may not reflect the actual response of plant roots, when grown in heterogenous growth substrate, as soil or peat, where factors, such as nutrient availability, and the composition and distribution of microorganisms may be highly influenced by the temperature.

5. Conclusion and future directions

The work of the present thesis was focused on the effects of low night temperature (LNT) on plant physiological responses in order to find the primary limiting factors, which may influence growth and maintenance of floricultural crops. The results are discussed in relation to an optimisation of climate control in greenhouse production aiming at saving energy.

In the present work it was found, that starch accumulated in the leaves of chrysanthemum, when the average night temperature was relatively low (8°C) in climate chamber studies, and in greenhouse studies (reaching 13°C) (Kjær *et al*., Paper I; Kjær *et al*., Paper III). The results of the controlled climate experiment indicated, that one of the main limitations to plant growth and development at LNT are due to the plants being restricted by sink limitation during the night. In other words, starch accumulated in the leaves, because there was an imbalance in the amount of absorbed energy expressed as the amount of carbohydrates from photosynthesis, and the ability of the plants to export the carbohydrates to plant organs in need of carbohydrates. The results were supported by the results of the second experiment (Kjær *et al*., Paper II), where increased root zone temperatures did not increase the carbohydrate export from the leaves, as the expected increase in sink demand of the roots had no effect on the phloem loading process in the leaves. The results support the hypothesis, that root activity and growth is more or less regulated by shoot demands (Castle *et al*, 2006; Bassiriad, 2000), and that the carbohydrate export is restricted at temperatures below 10ºC, because of a conformation of the endoplasmic reticulum, which limit phloem loading (Gamalei *et al*., 1994).

It was demonstrated, that low night temperatures of 12°C and 8°C did not affect plant DM production of chrysanthemums, when the dark period was between seven and ten hours long (Kjær *et al*., Paper I; Kjær *et al*., Paper III). These results suggest, that chrysanthemums may be produced at lower night temperatures during the long day period, than currently used in this species. However, the DM production of the leaves increased, instead of in stem and root DM, because of the increased starch accumulation. Furthermore, the starch accumulation occurred on the expense of an investment in leaf initiation and leaf expansion (Kjær *et al*., Paper I). LNT also decreased CO_2 assimilation in the leaves, and there was negative linear relation between CO_2 assimilation and starch accumulation in the uppermost fully expanded leaves, which confirmed that accumulated carbohydrates is related to an end-product limitation of photosynthesis, which has also been seen other plants (Goldschmidt and Huber, 1992). Root growth, root morphology, and plant NO_3^- uptake was only slightly affected, which suggested that temperatures down to 8°C in the root zone have marginal effects on the roots of chrysanthemums, when grown in containers with low volume, and a high availability of nutrients.

At the present stage, it is not known whether, chrysanthemums, or plants in general, are able to use the accumulated starch at a later stage in plant development or in the post harvest phase. However, it is most likely, as research have shown that starch accumulated during the day, disappear from the leaves during the night (Stitt *et al*., 1978; Lin *et al*., 1988; Gerhardt *et al*., 1987; Miskell *et al*., 2002), and when cuttings of chrysanthemum are

stored in environments with low light (Druege *et al*., 2000). On the basis of this knowledge, it is suggested, that if low night temperatures in the long day period, are followed by higher or "normal" night temperatures during a following short day period, then the increased leaf starch content may provide the plants with an increased capacity to develop larger, and even more flowers. However, as it is known that plants may acclimate to a long term exposure to low night temperatures by having respiratory rates that equal the rates at higher temperatures (Lawrence and Holaday, 2000; Atkin and Tjoelker, 2003), the following increase in temperature may result in an increase in maintenance respiration, which may cause the stored carbohydrates to be lost quickly in respiration. In support of this, Druege *et al*. (2004) reported that the increased leaf starch content, which was an effect of nitrogen deficiency in pelargonium, largely disappeared within the first week in cuttings, which were harvested and used for immediate rooting, without promoting the cuttings with more roots in comparison to treatments were the leaf starch content was lower. In future studies on the growth of floricultural crops at reduced night temperatures, it needs to be clarified whether plants needs a gradual increase in temperature in order to use the stored carbohydrates later in plant development or in the post harvest phase.

Starch accumulation in the leaves of chrysanthemum was more pronounced in plants grown in climate chamber, in comparison with plants grown in a greenhouse under fluctuating climate conditions (Kjær *et al*., Paper I; Kjær *et al*., Paper III). This effect was possibly caused by a combination of different factors. These factors may be differences in light quality and quantity, humidity, the constant temperature and wind, and therefore also the vapour-deficit (VPD) in the climate chambers, and the difference in growth substrates used in the experiments of this thesis. Furthermore, while the night temperature was maintained almost constant in the climate chambers, the greenhouses showed moderate fluctuations both in the short and in the long term. Thus it may also be suggested that less starch is accumulated, or that more starch is degraded during the day in climates, with fluctuating light and temperatures. That starch may be degraded during the day is not a general assumption in the literature, as starch degradation is mainly thought to occur during the night, and to be regulated by the length of the preceding photoperiod (Zeeman *et al*., 2007). However, Fondy *et al*. (1989) showed that starch accumulation stopped, and starch degradation started in plants of bean and sugar beat, when the light level was below a threshold rate, likely in response to the low photosynthetic rate at this light level. These results suggests that starch degradation may occur at low light intensity during the day in greenhouses, and this may explain in part the difference in leaf starch content of plants grown in climate chamber and in a greenhouse.

In this study, starch accumulation occurred in the plants as an effect of reduced night temperatures during the whole dark period. However, whether it is possible to obtain similar effects, when the temperature is low in shorter periods, or only during some part of the night needs to be clarified, in order to understand whether the effect is present in a dynamic climate where night temperatures are not artificially reduced, as in the greenhouse climate used in Kjær *et al*. (Paper III). Furthermore, it needs to be clarified whether the starch accumulation and the effects on plant morphology occur as an effect of the total temperature integral or the average temperature (AT), or whether it is possible to avoid the

negative effects of the reduced night temperatures by maintaining a closer connection between the day and night temperature, irrespective of the temperature integral and AT.

The present results document, that greenhouse production of some floricultural crops can be done with lower night temperatures in the vegetative stage, than presently done. However, the minimum night temperature is highly species-dependent, and the starch accumulation may have and influence on plant morphology. To improve the use of low temperatures in greenhouse production, it is hypothesised, that the night temperature needs to be balanced in relation to the temperature and irradiance of the preceding day, in order to obtain a balance between the carbohydrates assimilated in photosynthesis, and the ability of the plants to transport and use the carbohydrates. Many descriptive and explanatory models have been developed in order to model DM production of floricultural crops. However, most of the current models are largely based on plant photosynthesis, where plant photosynthesis is optimised in relation to the irradiance, by adjusting temperature and CO_2. The models are purely source driven models, and do not include knowledge about the ability of the plants to use the carbohydrates assimilated in photosynthesis. In recent years, models of DM partitioning between different organs in the plants have been developed; however, these models are often species-specific, and not integrated with the photosynthesis models (Marcelis *et al*., 1998). It is suggested that knowledge about the effect of low temperature on carbohydrate metabolism and transport in periods when the light is low and during the night, may increase our understanding of how carbohydrate assimilation are connected to carbohydrate transport and use. This may contribute to the development of a combined source-sink model.

A large amount of knowledge about plant transport processes and the use of nutrients and carbohydrates in response to temperature are found and one of the challenges in the future work on optimisation of the dynamic climate control system is to use more of this information. This will improve our predictions in future experiments and make the design process easier. The goal is an expansion of the dynamic climate control system in order to include not only photosynthesis, but also models of assimilate transport within the plant, and use of carbohydrates in major plant sinks at different temperatures. This may improve the climate control in the greenhouses, and thereby improve the use of the energy applied to the production systems.

Paper I

Low night temperatures change whole-plant physiology and increase starch accumulation in *Chrysanthemum x morifolium*

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Low night temperatures change whole-plant physiology and increase starch accumulation in *Chrysanthemum x morifolium*

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SUMMARY
Overnight changes in carbohydrate levels and the uptake of $^{15}NO_3^-$, photosynthesis, and plant morphology were investigated in *Chrysanthemum x morifolium* with the aim of identifying the primary limiting factor to influence plant growth and maintenance at low night temperatures. Plants were grown in long day conditions in an aerated nutrient solution culture system placed in three identical climate chambers. Night/day temperature treatments were 18º/18ºC (control), 12º/18ºC, and 8º/18ºC, respectively. *Chrysanthemum x morifolium* had the same dry matter (DM) production in the three treatments; however, plants grown in the low night temperature treatments formed fewer leaves and the total leaf area decreased. In contrast, low night temperatures increased leaf DM content on the expense of root DM content. This was in part explained by starch accumulation, which occurred, because starch synthesised during the photoperiod did not disappear during the dark period. Low night temperatures decreased the N concentration of the plants; however, the reduction was limited and in part, explained by the above-mentioned starch accumulation in the leaves. The daily plant NO_3^- uptake was not affected by the low night temperatures, although the NO_3^- uptake rate was lower during the night when plants were grown at 8ºC. Furthermore, low night temperatures had no effect on stem length, which was in contrast to the earlier literature. No explanation could be found, however the short duration of the experiment might explain the missing effect. The present results provide new information on the limits in growing chrysanthemum and other floricultural crops in a dynamic climate-control system with low temperatures during the night and in other periods where plants are less active.

Increasing concerns about climate change and economic considerations in recent years have increased pressure on the greenhouse industry to reduce energy consumption and CO_2 emissions generated by heating. An energy-reducing dynamic climate control system, based on a model system for leaf photosynthesis, was described by Aaslyng *et al*. (2003). In this system, temperature and CO_2 were controlled according to natural irradiance and allowed to vary considerably more than under a standard climate. Plant production was generally maintained, indicating the beneficial outcome of saving energy by allowing the temperature to decrease during the night, and in other periods when plants were less active, but only if plant production was optimised when irradiance was high. The amount of energy saved in a dynamic climate control system depend strongly on the night temperature required by a given plant species. Lund *et al*. (2006) recently demonstrated that it is possible to produce a heat-demanding plant (*Hibiscus rosa-sinensis*) in a system where the temperature is allowed to drop to a minimum set point of 15ºC.

For cold-tolerant chrysanthemum, suboptimal temperatures also have a significant influence on plant development, especially flower initiation and flower development, which can be delayed (Van der Ploeg and Heuvelink, 2006). In studies by Hansen *et al*. (1996), it was demonstrated that it is possible to increase biomass production in *Chrysanthemum x morifolium* when exposed to suboptimal temperatures. However, the effects of temperature on biomass production in chrysanthemums are contradictory, and depend, in part, on cultivar, but also on interactions between temperature and other growth conditions, such as light intensity (Van der Ploeg and Heuvelink, 2006).

Detailed studies on the effects of suboptimal temperatures on carbohydrate metabolism have been performed in many plant species. Studies on *Gossypium hirsutum* (cotton) and *Arabidopsis thaliana* have shown that the diurnal turnover of leaf carbohydrates, especially starch, can be reduced at low temperatures (Warner *et al*., 1995; Strand *et al*., 1999). The mechanism proposed was a chilling-induced inhibition of phloem-export, leading to an accumulation of carbohydrates in the leaves, possibly facilitated by a reduction in phloem loading (Gamalei *et al*. 1994). This accumulation of starch at low temperatures is often related to a down-regulation of photosynthesis (Goldschmidt and Huber, 1992; Warner *et al.,* 1995).

Plant NO_3^- uptake during the night constitutes between 30 - 40% of the daily uptake in tomato and soybean (Le Bot and Kirkby, 1992; Delhon *et al*., 1995). Studies on the relationship between night temperature and NO_3^- uptake have not yet been performed; however, Rufty *et al*. (1989) showed that diurnal changes in NO_3^- uptake depend on current carbohydrate availability to the roots, which suggest that the decreased export of carbohydrates to the roots at low night temperatures (LNT) will decrease NO_3^- uptake during the night. The relationship between temperature and nutrient uptake was studied in *Zea mays* by Engels *et al*. (1992) and Engels and Marschner (1996) who showed that the uptake and xylem transport of macronutrients (N, K, Ca), but not micronutrients (Mn, Zn), were more related to shoot demand, than to temperature. These studies suggest that decreased carbohydrate export to the roots during the night may decrease NO_3^- uptake in plants grown at a LNT. However, as a LNT will not influence shoot demand for nutrients during the day, the overall effect on plant NO_3^- uptake might be limited.

Intensive research has been performed on chrysanthemum cultivars to elucidate the effects of LNT on growth and development, in order to breed new cold-tolerant cultivars (van der Ploeg

et al., 2007). However, knowledge on which physiological factors limit plant growth and maintenance remains slight. Furthermore, LNT is not a very well-defined term. In this study LNT is defined as temperatures below the optimum temperature range of a specific plant species or cultivar, but above temperatures at which plant growth is expected to stop, or to show large limitations.

The aim of the present study was to find the primary limiting factor which influences the growth and maintenance of chrysanthemum when grown at LNT. Based on evidence from other plants, we hypothesised that a LNT may increase starch accumulation, decrease leaf area, nutrient transport and photosynthesis, but not affect DM production of the chrysanthemums. In two experiments, we examined this hypothesis by studying the effects of a LNT on the vegetative *Chrysanthemum x morifolium*, cultivar 'Coral Charm'. In the first experiment, we studied the effects of LNT on plant growth and *in situ* CO_2 assimilation. In the second experiment, we stu-died the effect of LNT on overnight changes in carbohydrate levels and the uptake of $^{15}NO_3^-$.

MATERIALS AND METHODS

Experimental design

Plants were grown in aerated nutrient solution cultures for 4 weeks in three identical climate chambers (mb-teknik, Brøndby, Denmark) each equipped with nine 400 W HQI lamps. The photoperiod was 13 h 20 min, and the mean photon flux density (PPFD) was 430 μmol m^{-2} s^{-1} measured with a quantum sensor (Skye Instruments, Llandrindod Wells, United Kingdom) above plant canopy. During the first and last 40 min of the photoperiod, the light intensity was slowly increased and decreased to simulate dawn and dusk. At dawn the light level was regulated in steps each 20 min with a light intensity at 100 – 130, 260 - 340, and finally 450 - 550 μmol m^{-2} s^{-1}. At dusk, the procedure was reversed. The set point for relative humidity in all three chambers was 70%, and the dark period lasted for 10 h 40 min.

During the first week, plants in all three chambers were acclimated to climate chamber conditions at 18ºC. In the following 3 weeks, Night/day temperature treatments were 18º/18ºC (control), 12º/18ºC and 8º/18ºC respectively. To test that the temperature set points were being reached, temperatures were monitored with a datalogger (Campbell Scientific CR10, North Logan, Utah, United States) with four thermistor sensors (Betatherm NTC 100K6, Shrewsbury, United States) in each chamber. One sensor was placed above the plant canopy, and three sensors were submerged in the nutrient solution of the containers.

TABLE I

Mean values of average day and night temperatures measured in three climate chambers with different set points, and in three separate containers with 8 l nutrient solution. The temperature was measured at 1 min intervals.

Experiment	Treatment	Day temperature		Night temperature	
		Air	Solution	Air	Solution
	(ºC/ºC)	(ºC)	(ºC)	(ºC)	(ºC)
1	18/18	18.4 ± 0.2	18.6 ± 0.2	17.8 ± 0.2	18.1 ± 0.2
	18/12*	17.2 ± 0.5	16.6 ± 1.5	11.5 ± 1.2	12.5 ± 1.8
	18/8	18.8 ± 0.5	18.5 ± 2.7	9.2 ± 1.2	10.2 ± 2.5
2	18/18	18.7 ± 2.3	18.8 ± 0.5	18.8 ± 0.5	18.2 ± 0.3
	18/12	18.4 ± 1.5	18.5 ± 1.1	12.2 ± 1.3	11.9 ± 1.3
	18/8	18.3 ± 1.8	18.1 ± 1.9	8.5 ± 2.2	9.9 ± 2.3

* Plant data from the treatment was excluded from analysis

The experimental design and harvest procedures were identical for the two experiments, although the timing and number of harvests differed. The first experiment included two harvests at 2 and 4 weeks after exposure to LNT; whereas the second experiment only included one harvest after 3 weeks exposure. In addition, the 12ºC treatment in the first experiment was excluded from the data analysis because the day temperature of the climate chamber only reached a mean value of 17.2ºC ± 0.5 (Table I). It was decided to exclude the treatment from the experiment, because day temperatures are known to have a larger impact on plant growth and morphology than night temperature.

Plant material and nutrient solution

Cuttings of *Chrysanthemum x morifolium* Ramat. - cultivar 'Coral charm' were selected for vigour and uniformity, and propagated in a 0.25 strength nutrient solution (see below) under long day (LD) conditions in a greenhouse (18 h photoperiod) to ensure vegetative growth. After 3 weeks, seedlings were transplanted into an aerated nutrient solution of the following composition: 48.3 mM $Ca(NO_3)_2$, 9.6 mM $NaNO_3$, 8.6 mM $Mg(NO_3)_2$ 6 H_2O, 0.6 mM KCl, 1.8 mM Fe-EDTA, 34.3 mM KNO_3, 6.5 mM KH_2PO_4, 9.2 mM K_2SO_4 and 3.7 mM $MgSO_4$ 7 H_2O plus micronutrients. NH_4^+ was excluded from the nutrient solution to prevent temperature-dependent discrimination between NO_3^- and NH_4^+. The pH was measured and corrected to pH 5.8 with NaOH and HNO_3 every second day. In the beginning, the pH was almost constant, whereas it decreased daily by 1.5 at the end of the experiment, possibly due to increased root activity and the larger root systems. Plants were grown in rectangular plastic containers (27 x 17 cm) with 8 l nutrient solution, each containing three plants. Plant stems were placed in slits in a circular styrofoam sheet, which was fastened in an open plastic tube with a diameter of 9 cm and a length of 18 cm. The tubes were fastened in the container lit and when closing the container, the tubes were submerged in nutrient solution, which avoided tangling of roots from the different plants. The containers were assigned at random to the three temperature treatments. Eighteen containers were included in the first experiment, and nine containers in the second experiment.

The CO_2 assimilation

The CO_2 assimilation was measured *in situ* using a portable open gas-exchange system incorpo-rating infrared CO_2 and water vapour analyses (CIRAS-1, PP-systems, Hitchin, United Kingdom). CO_2 assimilation was measured on four plants from each of the two treatments in the first experiment, and repeated twice during the experimental period, after 1 and 3 weeks of LNT, respectively. After 1 week, The initial CO_2 assimilation measurements was made on leaves developed before the experiment, while the later measurements were on leaves developed during the experiment. The upper-most third fully-expanded leaf was clamped in a leaf cuvette (1.8 cm in diameter) with temperature control. Measurements lasted from 4 to 8 hrs after the end of the dark period at 350 µl l^{-1} CO_2, 18ºC and under the light and humidity conditions of the climate chambers.

Harvest procedure and plant morphology

At each harvest, in both experiments, roots were separated from shoots, rinsed in demine-ralised water and dried between paper towels. Fresh weight (FW) was determined and leaf and

root samples for carbohydrate analysis were frozen directly in liquid nitrogen and stored at -80ºC. Weighed root samples (200 – 300 mg) were taken for determinations of root length and surface area. Leaves were counted and total leaf areas were measured on a LI-COR portable leaf area meter (LI-3000; Lambda Inst. Corp., Lincoln, Nebraska, United States). Leaves, stems and roots were dried at 70ºC for 24 h and their dry weight (DW) was determined. The root samples were distributed evenly in a transparent tray and scanned on a flat bed scanner. The scanned image was analysed by in an image analysis program (WinRhizo V 5.0A; Regents Instruments Inc., Quebec, Canada).

Overnight uptake of $^{15}NO_3^-$

In the second experiment, a study of overnight $^{15}NO_3^-$ uptake was performed together with a study of changes in carbohydrate levels in the four upper-most fully expanded leaves. Before adding $^{15}NO_3^-$ to the nutrient solution, one randomly-chosen plant from each of the three replicate containers within a chamber was harvested 1 h 40 min before the onset of the dark period, as control plants. The nutrient solution of the containers was then replaced with a similar nutrient solution containing 3.1 atom% $^{15}NO_3^-$. 4.2 mM $Na^{15}NO_3^-$ together with 5.4 mM non-enriched $NaNO_3^-$ replaced the 9.4 mM non-enriched $NaNO_3^-$, which was present in the original nutrient solution at the beginning of the experiment. After 1 h 40 min in light and 10 hours in the dark (dark period) and after an additional 12 h day (photoperiod), three plants from each chamber were harvested and dried at 70°C.

Dried shoot and root material was finely ground (< 0.25 mm) and analysed for ^{15}N and total-N by elemental analyser isotope ratio mass spectrometry in a commercial laboratory (Is-Analytical Ltd., Sandbach, United Kingdom). Excess ^{15}N enrichment of plant material after the dark period, and after the following light period were calculated by subtraction of the natural ^{15}N enrichment of the plant material, determined at the first harvest before addition of $^{15}NO_3^-$ to the nutrient solution.

TABLE II

Growth and morphology of Chrysanthemum x morifolium *grown at a day temperature of 18ºC and different night temperatures in two experiments*

Experiment	Harvest (week)	Night temp. (ºC)	Plant DM (g plant^{-1})	Leaves DM (g leaves^{-1})	R:S ratio (g g^{-1})	Leaves per plant (number)	Total leaf area (cm^2)	Root length (m plant^{-1})
1	2	18	5.18	3.16 a	0.28 a	34 a	399 a	5.1
		8	5.13	3.45 b	0.21 b	26 b	309 b	3.2
	4	18	15.51	7.97 a	0.28 a	67 a	1318 a	9.6
		8	16.45	8.39 b	0.22 b	51 b	941 b	5.2
2	3	18	6.36	3.29 a	0.28 a	49 a	581 a	5.7
		12	6.45	3.77 b	0.26 b	35 b	489 b	5.8
		8	6.84	4.20 c	0.22 c	34 b	450 b	5.4

Different letter(s) indicate differences between treatments at the particular harvest ($P < 0.05$)
Values of treatments are different between harvests ($P < 0.05$)

Diurnal changes in carbohydrate levels

The upper-most four fully-expanded leaves and additional root materials were sampled for analysis of diurnal changes in soluble sugars and starch. Samples were taken from three different plants in each treatment at each time-point, to follow the whole light/dark cycle. The four leaves from each sample were divided into two sub-samples; first and third leaf (sample one), and second and fourth leaf (sample two). The root sample was treated as one sample. Prior to analysis, samples were freeze-dried and ground in liquid nitrogen. Concentrations of hexoses (glucose + fructose) and sucrose were determined by HPLC (Hewlett Pacard 1047A; Waldbronn, Germany) as described by Liu *et al*. (2004). Starch was determined in the pellets remaining after extraction of the soluble sugars. The pellets were dried in a vacuum centrifuge and the starch was gelatinised by boiling for 1 h with a thermo-stable amylase (Termamyl; Novozymes, Bagsvaerd, Denmark) in 5 mM sodium dehydrogen phosphate buffer, pH 6.0. After centrifugation, the gelatinised starch in the supernatant was hydrolysed further with amyloglucosidase (Roche Diagnostics, Basel, Switzerland) in 50 mM sodium acetate buffer and 15 mM $MgCl_2$, pH 4.6, at 55°C for 1 h. The extracts were purified by anion exchange Sephadex QAE-A-25 (Pharmacia Biotech; Uppsala, Sweden) chromatography. The columns (1.0 ml volume) were pre-equilibrated with 0.5 ml sodium formate and washed with 50 ml 0.05 M sodium formate before sample application. The eluates were evaporated to dryness and re-dissolved in 0.5 ml water, and glucose concentrations were analysed by HPLC.

Data analysis

Mean temperatures in the climate chambers were calculated by the SummaryBy function in the software package R Release 2.2.1. Differences between temperature treatments, in biomass production, plant morphology, photosynthetic carbon assimilation, $^{15}NO_3^-$ uptake and carbohydrate distribution were analysed by linear mixed effects model allowing for nested random effects using software package R Release 2.2.1 *(http://www.r-project.org).*

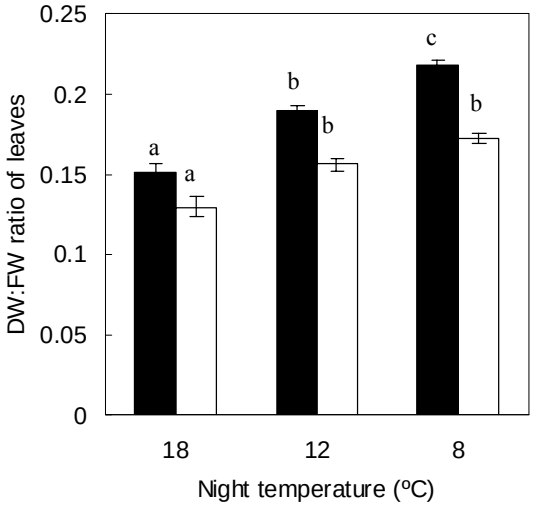

FIG. 1

Dry weight: fresh weight ratios of chrysanthemum leaves when plants were grown at a day temperature of 18ºC and different night temperatures. Plants were harvested after 4 weeks. DW:FW ratio of leaves (black bars). DW:FW ratio of leaves after subtraction of the starch content (white bars). Bars represent means ± SE of nine replicates. For each replicate, different letters indicate difference between treatments ($P < 0.05$).

RESULTS

Plant growth

LNT did not affect the total dry matter (DM) production of plants in any of the experiments, but leaf DM production increased, imposing a decrease in the root: shoot ratio (R:S) (Table II). Plants grown at a LNT formed less leaves, and their total leaf area was smaller. However, stem length was not affected, neither was total root length, root area, root diameter or root volume (Table II;

results not shown for all parameters).

LNT increased DM% (The DW:FW ratio), and when starch was subtracted from total leaf DM, there was no significant difference between plants grown at 8°C or 12°C (Figure 1). The relation between the DM% and starch concentration, in the upper-most fully expanded leaves of chrysanthemum, indicated that the increased DM% in plants grown at a LNT, could be ex-plained in part by an increase in the starch concentration in the same leaves (Figure 2).

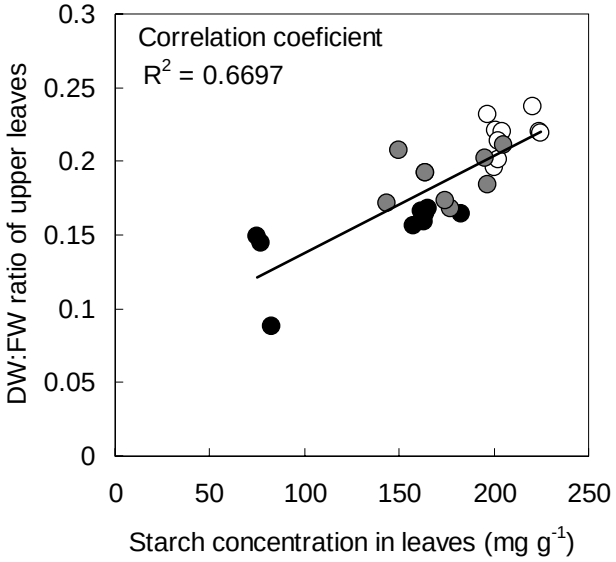

FIG. 2
Relationship between the DW:FW ratio of chrysanthemum leaves and the concentration of starch in the leaves of plants grown at a day temperature of 18ºC and at three different night temperatures: 18ºC (black circles), 12ºC (grey circles), 8ºC (white). The correlation coefficient (R^2) is based on results from all three treatments.

Carbohydrate levels and starch accumulation

Diurnal changes in the concentration of nonstructural carbohydrates, in leaves of chrysanthemum, were significantly decreased in plants grown at a LNT (Figure 3A). The lack of an overnight degradation of starch, imposed an increase in the overall starch concentration of the upper-most fully expanded leaves in plants grown at a LNT; even though, the accumulation of starch during the following day was low compared to plants grown at 18ºC during the night. At the end of the dark period, the starch concentration was about twice as high in plants grown at 12ºC in comparison with plants grown at 18ºC. For plants grown at 8ºC, the starch concentration was even further increased. Starch did

TABLE III

The effect of night temperature on CO_2 assimilation in the third fully-expanded leaf of Chrysanthemum x morifolium *plants measured 4 h after the end of the dark period at ambient CO_2 concentration (350 µl l^{-1}), 18ºC and under the light conditions in the climate chambers (430 µmol m^{-2} s^{-1}). Data are means of ten replicate measurements on each of four plants per treatment.*

Measurement time (weeks)	Night temperature (ºC)	CO_2 assimilation (µmol m^{-2} s^{-1})	Intercellular CO_2 (Ci) (µl l^{-1})	Stomatal conductance (Gs)
1	18	12.5 a	353.7 a	124.3 a
	8	11.4 b	375.3 a	116.4 a
3	18	12.7 a	250.3 b	90.0 b
	8	11.9 b	276.4 b	81.8 b

Different letter(s) indicate differences between treatments and harvests ($P < 0.05$)

not accumulate in the roots of chrysanthemum, but starch levels were lower prior to the dark period in plants grown at a LNT (Figure 3B). The concentration of soluble sugars in the leaves of chrysanthemum was 90% lower than starch concentrations, and there was a trend towards decreased concentrations in plants grown at a LNT (Figure 3C). The concentration of soluble sugars in the roots was twice as high as in the leaves; however, there was no significant diurnal changes (Figure 3D).

The CO_2 assimilation

The CO_2 assimilation ($\mu mol\ m^{-2}\ s^{-1}$) was significantly reduced by LNT, both in leaves which were formed before the experiment and in leaves formed during the experiment (Table III). The values of stomatal conductance (g_s) and intercellular CO_2 concentration (C_i; $\mu l\ l^{-1}$) were significantly lower at the second measurement time compared to the first measurement time; however, this did not influence the rate of CO_2 assimilation. The decrease in CO_2 assimilation at a LNT of 8ºC was accompanied by a non-significant increase in C_i and a decrease in g_s. However no relation was found between CO_2 assimilation and C_i or g_s (results not shown).

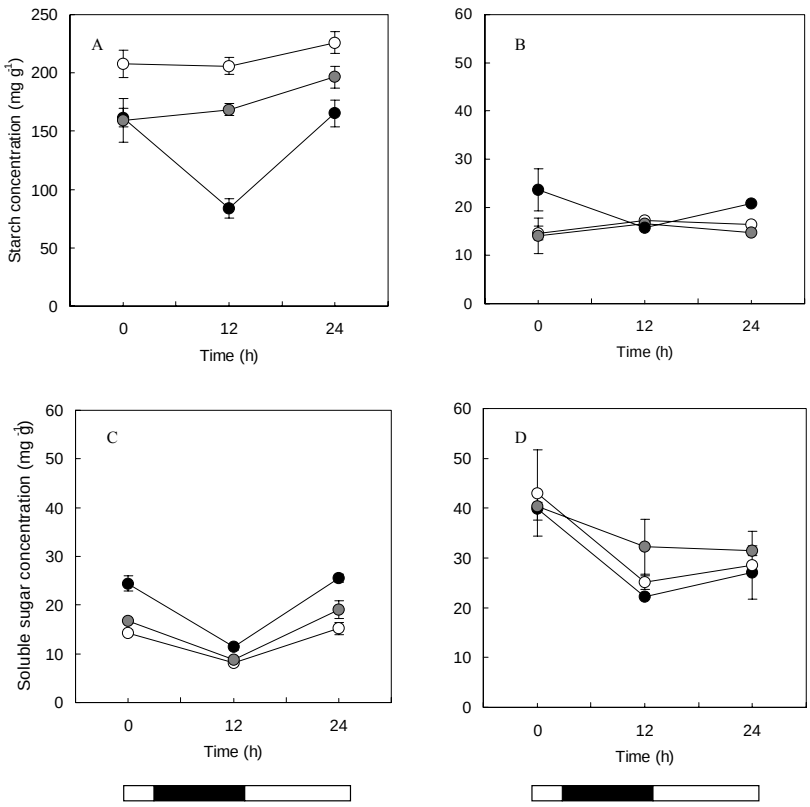

FIG.3
Diurnal variation in carbohydrate concentrations in the four upper-most fully expanded chrysanthemum leaves when plants were grown at a day temperature of 18ºC and three different night temperatures: 18ºC (black circles), 12ºC (grey circles) and 8ºC (white circles). Starch concentrations in shoots (A), and roots (B). Total concentration of soluble sugars including fructose, glucose and fructose in shoots (C), and roots (D). The black and white bar at the bottom illustrates the dark and light periods. Bars represent ± SE of three replicates.

Nitrogen (N) content and patterns in NO_3^- uptake and transport

The amount of N in the nutrient solution was 1.8 g N per container at the beginning of the experiment. At the end of the experimental period of 4 weeks, the total N content of the three plants from each container as approximately 900 mg N. It was therefore assumed, that the availability of N in the nutrient solution, did not limit plant N uptake. However, a LNT decreased the N-concentration in the shoot, even when the estimated starch content of the leaves was subtracted from the total leaf DM (Figure 4).

Labelled N as $^{15}NO_3^-$ was made available to the plants within a limited period of 24 h. The atom% of ^{15}N in the plants harvested prior to addition of $Na^{15}NO_3^-$ to the nutrient solution was 0.3687 and close to the naturally occurring atom% of ^{15}N. After the dark period, all plants had taken up excess $^{15}NO_3^-$; however, the content was significantly lower in plants grown at 8ºC in both roots and shoots than in the two other treatments (Figure 5A, B). After the following light period, there was no significant differences in the root-content of $^{15}NO_3^-$ between treatments, whereas the shoot content of ^{15}N was signifycantly lower for plants grown at 8ºC in comparison with plants grown at 12ºC and 18ºC during the night. The N uptake rate was significantly decreased during the night when plants were grown at 8ºC in comparison with the two other treatments. During the day no significant differences were seen between treatments (Table IV).

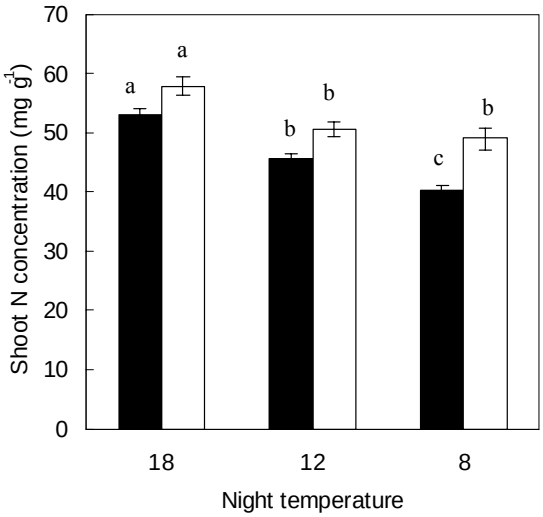

FIG. 4
Shoot N concentrations of *Chrysanthemum x morifolium* grown at a day temperature of 18ºC and three different night temperatures. Plants were harvested after 4 weeks. Shoot N concentration (black bars). Shoot N concentration after sub-traction of the estimated starch content from leaf DM. Bars represent ± SE of nine replicates. For each bar, different letters indicate difference between treatments ($P < 0.05$).

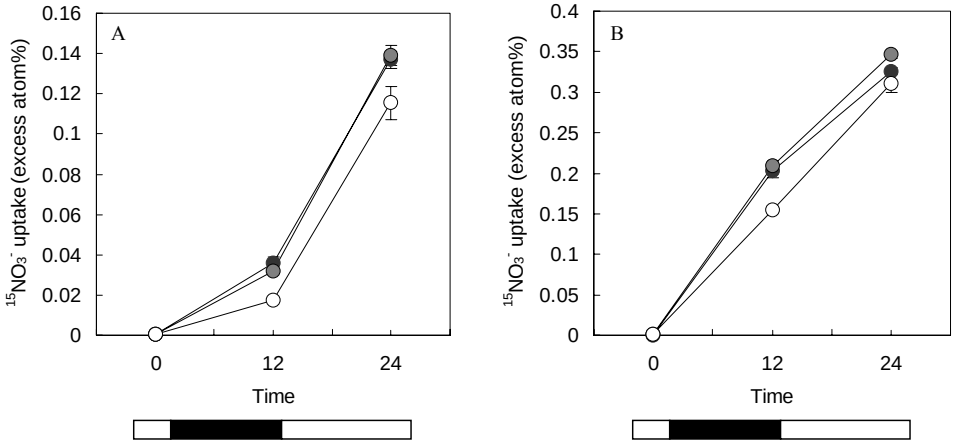

FIG. 5
The excess uptake of $^{15}NO_3^-$ in shoots (A) and roots (B) of *Chrysanthemum x morifolium* when plants were grown at a day temperature of 18ºC and three different night temperatures: 18ºC (black circles), 12ºC (grey circles) and 8ºC (white circles). Plants were supplied with a nutrient solution containing a 3.1 atom% of ^{15}N, 90 min before the beginning of the dark period. Natural ^{15}N enrichment of plant material measured at the first harvest (0 h) before addition of the new nutrient solution was subtracted from the results at the second (12 h) and third harvest (24 h). The black and white bars illustrate the dark and light period. Bars represent means ± SE of three replicates.

DISCUSSION

Low night temperatures (LNT) increased starch accumulation in leaves of *Chrysanthemum x morifolium*. Starch accumulation occurred, because the starch synthesised in the upper-most fully expanded leaves during the photoperiod, did not disappear during the dark period. This pattern confirms for the chrysanthemums what has been reported for a range of other plant species (Trethewey *et al*., 2000). In arabidopsis, the diurnal turnover of leaf carbohydrates was reduced when plants were shifted from 23ºC to 5ºC and after 10 d, leaves contained large stable pools of all carbohydrates including starch (Strand *et al*., 1999). In the present study, it was shown that starch accumulation in plant leaves can be achieved under much milder temperatures than used in Strand *et al*. (1999), and by lowering the night temperature only.

Plant growth

Whole plant DM production of vegetative propagated chrysanthemums did not respond to a LNT, which was also shown in others studies with vegetative, as well as in flowering chrysanthemums (Van der Ploeg and Heuvelink, 2006). In the present study, it was demonstrated that leaf DM increased significantly in chrysanthemums grown at a LNT, even after the estimated starch content of the leaves was subtracted from leaf DM. The results suggests, that only part of the increase in leaf DM can be explained by starch accumulation, or that the estimated starch content of all the leaves, which were calculated on the basis of results from the upper-most fully expanded four leaves, were underestimated. Our results indicate that the contradictory findings published on DM production in chrysanthemum cultivars, may be related to the ability of the cultivar to accumulate starch.

Stem length was not affected by LNT, which was in contrast to the earlier literature (Myster and Moe, 1995). No explanation can be found, although it is suggested that the short duration of the experiments may explain the missing effect.

Photosynthesis

In situ photosynthetic CO_2 assimilation in chrysanthemum leaves was slightly reduced when plants were grown at a LNT, which confirms results on other plant species (Warner *et al*., 1995; van Heerden *et al*., 2004). Reduced CO_2 assimilation in plants grown at LNT is often explained

TABLE IV

Whole-plant nitrogen relations of Chrysanthemum x morifolium *grown at a day temperature of 18ºC and three different night temperatures*

Night temperature (ºC)	N uptake (night) (mg g^{-1} h^{-1})	N uptake (day) (mg g^{-1} h^{-1})	N concentration (mg g^{-1})	N uptake (total) (mg day^{-1})	Night uptake (%)
18	0.14 a	0.47	53.7 a	31.52	33%
12	0.13 a	0.49	51.4 b	37.67	35%
8	0,07 c	0.50	49.3 b	35.82	21%

Different letter(s) indicate differences between treatments ($P < 0.05$)

All values are calculated on the basis of plant DW after the subtraction of the estimated starch content in all leaves from total leaf DM

as a feed-back regulation of carbohydrate metabolism, due to an increase in starch and a decrease in sucrose in the leaves (Goldschmidt and Huber, 1992; Warner *et al*., 1993). The relation between a down-regulation of CO_2 assimilation in response to carbohydrate metabolism was supported in the present study. However, the present down-regulation in CO_2 assimilation did not affect the DM production of the plants, which suggested, that a decrease in maintenance respiration during the night as an effect of LNT may have decreased a potential loss in DM of the plants, as also suggested by Parups and Butler (1982).

The NO_3^- uptake

It was demonstrated that the nightly NO_3^- uptake of vegetative chrysanthemums constitutes between 21-35% of the daily uptake depending on the night temperature, which confirmed other studies (Rufty *et al*., 1984; Le Bot and Kirkby, 1992). When chrysanthemum was grown at a night temperature of 8ºC, a significant decrease in the N concentrations of the plant was seen, even after starch was subtracted from leaf DM. This suggested that plants were either N-limited, limited by other nutrients (Engels and Marschner, 1996), or that storage compounds other than starch diluted the N concentration of the plant tissue.

The results demonstrated that LNT decreased the NO_3^- uptake rate of chrysanthemums during the night; however, plants compensated by having similar or slightly increased NO_3^- uptake rates during the day. There was no significant difference in N uptake between plants grown in the three treatments. Plants are known to have a highly adaptive N uptake system (Bassirirad, 2000). Stomatal closure, and a decrease in transpiration possibly explain the decrease in the N uptake rate during the night (Rufty *et al*., 1984).

The present results provide us with knowledge about some of the plant physiological responses, which explain plant responses to LNT. It is shown, that plants grown at LNT maintain their total DM production, possibly due to a decrease in respiration and an increase in the accumulation of carbohydrates in the leaves, which result in less carbohydrate available for formation of new leaves, leaf expansion and root DM production.

The decrease in export of leaf carbohydrates during the night was the most limiting factor to the chrysanthemums, and it indicates that there was in imbalance in the plant energy absorption in the form of carbohydrates from photosynthesis, and the use of carbohydrates for growth and maintenance during the night. This information is important, when improving the model system used in the dynamic climate control system. It indicates, that it might be important, to balance the incoming energy to the greenhouse, in the form of light, CO_2 and day temperature, with the night temperature, in order to obtain optimum conditions for the plant growth.

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Paper II

Root zone heating at 8ºC night air temperature does not decrease starch accumulation in *Chrysanthemum x morifolium*

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Root zone heating at 8ºC night air temperature does not decrease starch accumulation in *Chrysanthemum x morifolium*

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SUMMARY

It is of increasing interest to grow floricultural crops at lower night temperatures in order to save energy in greenhouse production; however, problems such as extended production time, and fewer flowers limits the use of low temperatures. These growth limitations may occur, because starch synthesised in the leaves during the day, is not exported from the leaves during the night. Root zone heating, during cold nights, may increase root growth and nutrient uptake. This may increase the root demands for carbohydrates, and the carbohydrate export from the leaves and reduce starch accumulation. To study this hypothesis, plants of *Chrysanthemum x morifolium* were grown in nutrient solution cultures at a day temperature of 20°C, and four combinations of night air temperatures (NAT) and night root zone temperatures (NRT) in a climate chamber experiment. The NAT/NRT temperatures of the four treatments were 8º/8ºC, 8º/20ºC, 20º/8ºC and 20º/20ºC, respectively. Plants grown at 8°C NAT had increased starch content in the leaves, and the starch content explained an increase in leaf DM, which occurred on the expense of stem and root DM. Furthermore, plants grown at 8ºC NAT had fewer leaves, reduced leaf area and shorter stems. Root zone heating of plants grown at the 8ºC NAT, did not affect total leaf number or leaf area, but the root length increased; however, this had no effect on NO_3^- uptake. Furthermore, root zone heating did not reduce the leaf starch accumulation and the increased leaf DM. The present results reject the hypothesis that root zone heating can be used to increase root demands for carbohydrates and increase carbohydrate export from the leaves at night.

A dynamic climate control system has been developed in order to save energy in greenhouse production of floricultural crops (Aaslyng *et al*., 2003). Energy is saved in the system, because temperature and CO_2 is controlled according to the incoming light, and because the temperature is subsequently allowed to vary considerably more than in a standard climate. Plant production is generally maintained at the same level in the system, in comparison with more traditional systems; although, the night temperature is relatively low. Knowledge about the ability of plants to cope with low night temperatures (LNT) is important, when optimising the dynamic climate control system, and root zone heating may be a beneficial tool in extending the optimal temperature range of the plants in relation to floricultural production. A LNT has been shown to delay plant development, and decrease the number of flowers at maturity in chrysanthemums (Carvalho *et al*., 2005). However, low night temperatures have also been shown to increase the relative growth rate (RGR), dry matter (DM) production and leaf DM (Hansen *et al*., 1996, Van der Ploeg and Heuvelink, 2006). Starch accumulation, explain in part the increase in leaf DM, when plants of *Chrysanthemum x morifolium* are grown at LNT in nutrient solution culture under climate chamber conditions (Kjær *et al*., accepted), illustrating that sink limitation could be one of the main limitations to plant development at LNT.

Root zone heating at LNT increase leaf area, flower bud diameter, and decrease time to flowering of chrysanthemums (Brown and Ormrod, 1980, Tsujita *et al*., 1981). The reasons for these responses are unknown, but increased capacity of the root system to take up nutrients, and maintain plant water status, may be important factors. In support of this, studies have shown that plant nutrient uptake is generally increased in response to soil warming, and one reason is an increased root uptake capacity (Bassirirad, 2000). Temperature sensitive processes, such as root respiration and hydraulic conductivity explain in part the observed changes. However, the sensitivity of plant NO_3^- uptake capacity has been shown to be related to the N-status of the plant. Lainé *et al.* (1993) demonstrated that NO_3^- uptake capacity was only sensitive to changes in root zone temperatures, when plants were N-starved. This was confirmed by Castle *et al*. (2006), who also demonstrated, that although NO_3^- uptake was not limited by a low root zone temperature, N was preferentially stored in the roots of *Trifolium repens* (white clover), because N-transport to the shoot was limited by low air temperature. Plant NO_3^- uptake during the night constitute up to 50% of the daily uptake (Rufty *et al*., 1984), which suggests, that changes in night air temperature (NAT), and night root zone temperature (NRT) may influence on NO_3^- uptake and transport to the shoot. However, in a former study, low night temperatures only had a small effect on uptake and transport of $^{15}NO_3^-$, possibly as a consequence of a high N-status of the plants, and because the plants compensated by having a higher NO_3^- uptake rate during the day (Kjær *et al*., accepted).

Root zone heating has been shown to increase the carbon exchange rate of leaves, and the transport of carbon to the roots of tomatoes (Hurewitz and Janes, 1983). However, the plants were grown at an ambient AT of 22ºC, and this may have contributed to the positive effect of root zone heating; possible, by increasing the temperature dependent phloem loading of carbohydrates (Gamalei *et al*., 1994). In other studies, it has been confirmed, that positive effects of root zone heating are more pronounced, when plants are not limited by low NAT (Gosselin and Trudel, 1986), and that the optimal root zone temperature may even increase the plants ability to cope with supraoptimal air temperatures (Thompson *et al*., 1998).

In this study, it was hypothesised that when the root is heated at 8°C NAT, increased root growth and nutrient uptake may lead to increased root demand for carbohydrates and thereby increased export of carbohydrates from the leaves. This may lead to increased root DM, and a lower starch accumulation in the leaves.

MATERIALS AND METHODS

Plant material and nutrient solution

Cuttings of *Chrysanthemum x morifolium* cultivar 'Coral Charm' were selected for vigour and uniformity and propagated in a ¼ strength nutrient solution (see below) under long day conditions in a greenhouse (18 h day and 6 h night) to ensure vegetative growth. After 3 weeks, seedlings were transplanted into the aerated nutrient solution of the following composition: 5.7 mM $NaNO_3$, 12.6 mM NH_4NO_3, 8.9 mM KCl, 0.7 mM Fe-EDTA, 47.6 mM KNO_3, 0.4 mM $(NH_4)^2SO_4$, 3.4 mM $(NH_4)H_2PO_4$, 2.6 mM KH_2PO_4, 5.8 mM $MgSO_4$ and micronutrients. The pH was measured and corrected to 5.8 with NaOH and HNO_3 every day. Initially, the pH was almost constant, whereas it decreased daily at the end of the experiment (1.5), possibly due to increased root activity and a larger root system.

Plants were grown in rectangular plastic containers (27 x 17 cm) with 8 l of nutrient solution, each containing three plants. Plant stems were placed in a slit of a circular styrofoam sheet, which was fastened in an open plastic tube with a diameter of 9 cm, and a length of 18 cm. The tubes were fastened in the container lit, and when closing the container, the tubes were submerged in the nutrient solution, and interaction between roots from different plants was avoided. In each treatment, six containers were connected to each other in a serial system with a thermal water bath (CB5, Heto, Copenhagen, Denmark). Each system contained 75 l nutrient solution in total, circulated with a flow of 11 ml s^{-1} by a water driven pump.

Experimental design

Plants were grown in aerated nutrient solution cultures for 4 weeks in two identical climate chambers (mb-teknik, Brøndby, Denmark) each equipped with 30 HQI-BT lamps (400 W). The photoperiod was 16 h 20 min and the mean photon flux density (PPFD) was 498 µmol m^{-2} s^{-1} measured with a quantum sensor (Skye Instruments, Llandridod Wells, United Kingdom) at plant level. During the first and last 40 min of the photoperiod, light was slowly increased and decreased to simulate dawn and dusk. At dawn, the light level was regulated in steps each 20 min with light levels at 130-140, 222-268, and finally 475 - 540 µmol m^{-2} s^{-1}. At dusk, the procedure was reversed. The set point for relative humidity was 70%, and the dark period was 7 h 40 min. During the first week, plants were acclimated to climate chamber conditions at 20ºC in both root zone and air all day. In the following 3 weeks the day temperature was 20ºC in both root zone and air, whereas the night temperature differed in all four treatments. The night temperature treatments were: 8°/8°C, 8°/20°C, 20°/8°C and 20°/20ºC (control) in air and root zone, respectively. Temperature was monitored with two sensors (PT100, Ametek, Alleroed, Denmark) in each treatment every 10 min, submerged in the nutrient solution using a datalogger (dataTaker Pty Ltd., Germany).

There was no difference in the mean air temperature during the day in the two climate chambers. When lowering the temperature during the night, the air temperature set point was reached within the first 15 min, and the mean NAT was 19.2ºC ± 0.4 and 8.0ºC ± 2.5 in the two

chambers, respectively. The temperature response of the nutrient solution was slower, and the 8ºC NRT was reached after two hours. The mean temperatures of the 8ºC NRT and the 20ºC NRT treatments did not differ significantly. The mean NRT was 19.4°C ± 0.6 and 9.7°C ± 2.5 in the two treatments, respectively. The mean root zone temperature during the day was 20.7°C±1.1 in all treatments.

The CO_2 assimilation and carbohydrate content.

The CO_2 assimilation was measured using a portable open infrared gas-exchange system (CIRAS-2, PP-systems, Hitchin, United Kingdom). CO_2 assimilation was measured on three plants from each treatment, and repeated every week during the experimental period. Leaf samples for carbohydrate analysis were instantly frozen in liquid nitrogen and stored at -80ºC, just after the gas exchange measurement. The initial CO_2 gas exchange measurements was made on leaves formed before the experiment, while the later measurements were on leaves formed during the experiment.

The third fully expanded leaf was clamped into a leaf cuvette (1.8 cm in diameter) with light, CO_2, humidity and temperature control. Measurements lasted from three to seven hrs after the end of the dark period at a photosynthetic flux density (PPFD) of 900 µmol m^{-2} s^{-1}, 1000 µl l^{-1} CO_2 and 20ºC.

Overnight uptake of $^{15}NO_3^-$ and carbohydrate content

After 3 weeks growth at different night temperatures, an overnight analysis of $^{15}NO_3^-$ uptake, and measurements of diurnal changes in carbohydrate levels, was performed on the plants. Before adding the $^{15}NO_3^-$ to the nutrient solution, three randomly chosen plants from each treatment were harvested (the control). The remaining plants were temporary placed in containers with nutrient solution, but no air supply, for 30 min. The lack of air supply may have caused oxygen limitations to the plants, as leaves quickly became slacken. The nutrient solution of all culture systems was replaced with a new nutrient solution containing 4.4 atom% of $^{15}NO_3^-$ of total N, 10 h before the start of the dark period. After the first 10 h (just before the dark period) three plants from each treatment were harvested. Thereafter, three plants were harvested after the dark period of 7 h 40 min, and after the following light period of 16 h, to provide a total of three harvest times plus the control. At each harvest, roots were separated from shoots, rinsed in demineralised water and dried between paper towels. Fresh weight (FW) was determined and leaf samples for carbohydrate analysis were frozen in liquid nitrogen and stored at -80ºC. Shoots and roots were dried at 70ºC for 24 h, and dry weight (DW) was determined.

Dried material of shoot and root was finely ground (< 0.25 mm) and analysed for ^{15}N and total-N by elemental analyser isotope ratio mass spectrometry in a commercial laboratory (Iso-Analytical Ltd., Sandbach, United Kingdom). Excess ^{15}N enrichment of plant material after the dark period, and after the following light period were calculated by subtraction of natural ^{15}N enrichment of the plant material, determined at the first harvest, before addition of $^{15}NO_3^-$ to the nutrient solution.

Carbohydrate analysis

The four upper-most fully expanded leaves were sampled for analysis of soluble sugars and starch at each harvest. Prior to analysis, samples were freeze-dried and ground in liquid nitro-

gen. Concentrations of hexose (glucose + fructose) and sucrose were determined on HPLC (Hewlett Pacard 1047A, Waldbronn, Germany), as described by Liu *et al*. (2004). Starch was determined in the remaining pellets, after extraction of soluble sugars, as described in Kjær *et al*. (accepted).

Growth analysis

During each week, plants were harvested for growth analysis. At each harvest, weighed root samples (200 – 300 mg FW), representative for the whole root system, were taken for determination of the root length and surface area. Leaves were counted, and leaf area was measured on a LI-COR portable leaf area meter (LI-3000, Lamda Inst. Corp., Lincoln, Nebraska, United States). Leaves, stems and roots were dried at 70ºC for 24 h, and their DW was determined. The root samples were distributed evenly in a transparent tray and scanned on a flat bed scanner. The scanned image was analysed in an image analysis program (Win-Rhizo V 5.0A; Regents Instruments Inc., Quebec, Canada).

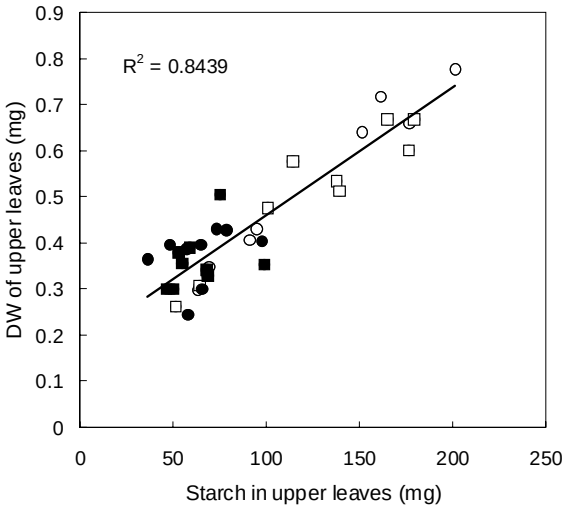

FIG. 1
Relationship between the dry weight (DW) of the four upper-most fully expanded leaves, and starch content in the same leaves of *Chrysanthemum x morifolium* grown at a day temperature of 20ºC and 4 combinations of night air temperature (NAT) and night root zone temperature (NRT). NAT of 20ºC (black symbols) or 8ºC (white symbols). NRT of 20ºC (squares) or 8ºC (circles). The correlation coefficient R^2 is based on results from all three treatments.

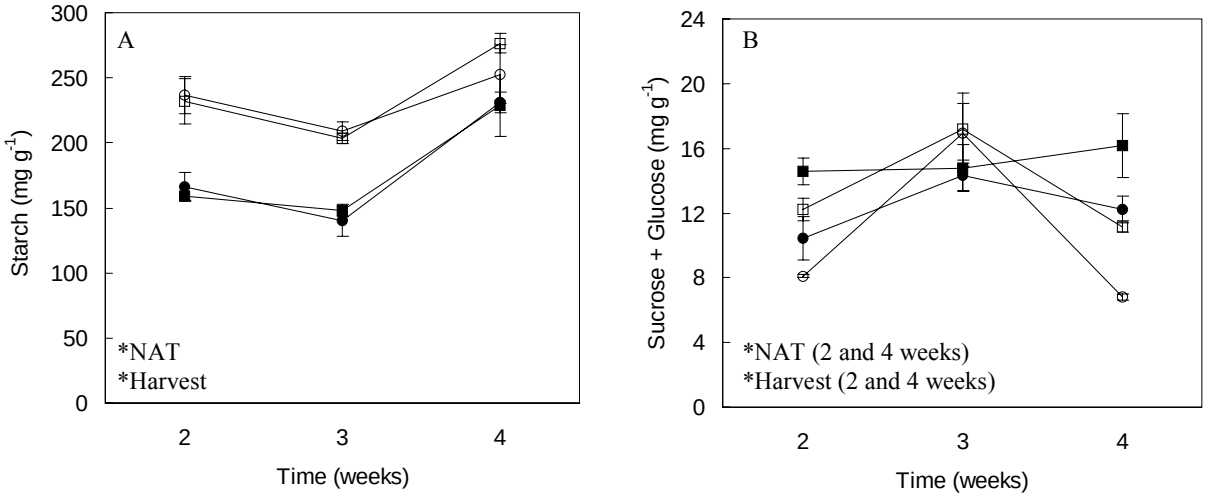

FIG. 2
Long term changes in carbohydrate concentrations in leaves of *Chrysanthemum x morifolium* grown at a day temperature of 20ºC and 4 combinations of night air temperature (NAT) and night root zone temperature (NRT). NAT of 20ºC (black symbols) or 8ºC (white symbols). NRT of 20ºC (squares) or 8ºC (circles). Starch concentration in leaves (A). Concentration of sucrose and glucose in leaves (B). (n = 3, * $P < 0.05$).

Data analysis

Differences across temperature treatments in biomass accumulation, morphology, photosynthetic carbon assimilation, $^{15}NO_3^-$ uptake and carbohydrate distribution were analysed by linear mixed effects model allowing for nested random effects using software package R release 2.4.1 *(http://www.r-project.org).*

RESULTS

Plant growth and morphology

Plant DM production was similar in all four treatments (Table I); although 8ºC NAT significantly increased leaf DM and decreased stem and root DM. Furthermore, 8ºC NAT decreased total leaf number and stem length, but not the total leaf area. Differences in NRT had no significant effect on these parameters, but 8ºC NRT decreased total root length.

The DM content of the leaves was linearly related to the starch content, indicating that the starch accumulation in these leaves explained the increase in leaf DM of plants grown at 8°C NAT (Figure 1).

TABLE I

Growth and morphology of Chrysanthemum x morifolium *grown at a day temperature of 20ºC and different nigh air temperatures (NAT) and night root zone temperatures (NRT).*

Harvest (week)	NAT (ºC)	NRT (ºC)	DW plant (g)	leaves (g)	stem (g)	root (g)	Leaves (no.)	Leaf area (cm^2)	Stem length (cm^2)	Root length (cm)
2	8	8	8.2[a]	5.6	1.7	1.0	28	465	19.3	203
		20	9.3	5.8	2.3	1.2	31	523	19.6	336
	20	8	8.4	5.0	2.2	1.3	34	564	21.3	275
		20	8.0	4.5	2.2	1.2	36	576	23.0	355
3	8	8	16.1	9.8	4.5	1.8	67	1,220	25.4	571
		20	16.6	9.8	5.0	1.7	64	1,278	27.3	968
	20	8	13.4	7.1	4.6	1.8	66	1,179	30.0	619
		20	16.9	8.9	5.9	2.1	77	1,546	29.0	1,026
4	8	8	29.2	18.3	8.2	2.6	95	1,941	31.7	1,199
		20	36.0	21.2	11.8	2.9	109	2,465	31.9	1,405
	20	8	30.7	15.1	11.8	3.8	126	2,631	33.7	1,177
		20	34.7	18.4	12.2	4.1	127	2,770	33.4	2,010
Factors		*df*	*P* value[b]							
Harvest		2	**	**	**	**	**	**	**	**
NAT		1		*	*	**	**		**	
NRT		1								**

[a]Each value is the mean of three replicates.

[b]*P* value for differences of means (Linear mixed-effects model).

** significant at $P < 0.01$, * significant at $P < 0.05$

Photosynthesis and carbohydrate levels

The 8ºC NAT reduced the maximum capacity for CO_2 assimilation in the leaves of Chrysanthemum significantly, and correspondingly heating of the roots at 8ºC NAT signifycantly increased the CO_2 assimilation, although the rate did not reach the levels measured in the treatments of 20ºC NAT (results not shown).

The 8ºC NAT significantly increased starch accumulation in the leaves, and the difference between the treatments was constant throughout the experiment (Figure 2a). Furthermore, there was a negative linear relation between the starch concentration in the leaves and CO_2 assimilation (Figure 3). The concentration of sucrose and glucose in the four fully expanded leaves of chrysanthemum was significantly decreased after 2 and 4 weeks by both 8°C NAT and 8°C NRT. However, the effect was opposite, although not significant, after 3 weeks, and possibly caused by the observed oxygen limitations at this harvest (Fig. 2b). Fructose concentrations were low, and no differences were seen between treatments (results not shown)

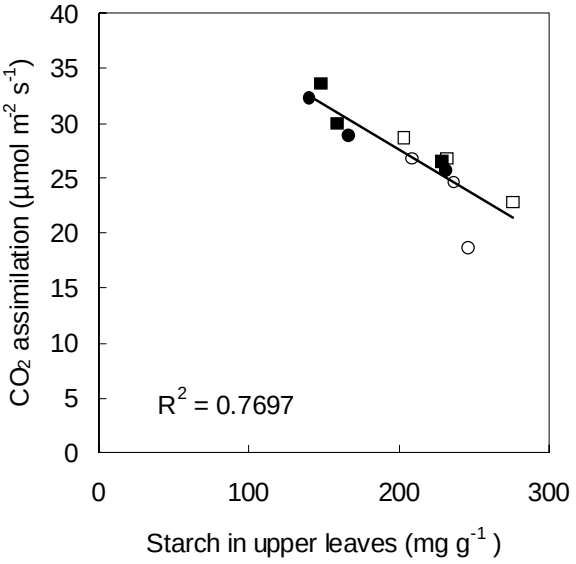

FIG. 3
Relationship between the CO_2 assimilation (µmol m^{-2} s^{-1}) and the starch concentration in the same leaves of *Chrysanthemum morifolium* grown at a day temperature of 20ºC and 4 combinations of night air temperature (NAT) and night root zone temperature (NRT). NAT of 20ºC (black symbols) or 8ºC (white symbols). NRT of 20ºC (squares) or 8ºC (circles). The correlation coefficient R^2 is based on results from all three treatments.

Diurnal changes in carbohydrate levels.

The concentration of starch was 25% higher in plants grown at 8ºC NAT, in comparison with plants grown at 20ºC NAT. The difference between treatments was constant throughout the diurnal cycle (Fig. 4a).

The effect of NAT on the concentration of soluble sugars (sucrose and glucose) in the young leaves did not significantly differ between harvest times (Fig. 4b). However, the results indicated that the concentration of sucrose and glucose increased during the night in plants grown at 8ºC NAT, whereas the concentration of soluble sugars decreased during the night in plants grown at 20ºC NAT. Differences in NRT did not change the diurnal pattern in the concentration of starch, sucrose or glucose, significantly.

Nitrogen (N) content and NO_3^- uptake

The 8ºC NAT decreased the N concentration of shoots after starch had been subtracted from the leaf DW, whereas NRT had no effect (Table II). However, NRT increased the total N content per shoot. The mean atom% of ^{15}N in the control plants, harvested before the addition of $^{15}NO_3^-$ was 0.3663 and 0.3667 in shoots and roots, respectively, and close to the naturally occurring atom% of ^{15}N. After 10 h light, all plants had taken up $^{15}NO_3^-$, and no significant differences were found in the excess ^{15}N in either roots or shoots between treatments (results not

shown). Nor, after the following dark, and light period, any significant differences in $^{15}NO_3^-$ uptake between treatments were seen; although, the uptake rate during the night was lower in plants grown at 8ºC NRT, in comparison with plants grown at 20ºC NRT (Table II). However, significant differences in total NO_3^- uptake rate were only seen between night and day, and not between treatments. At the 3 weeks harvest, the mean content of $^{15}NO_3^-$ taken up during one night was 24 mg/plant and the mean content of total N was 610 mg/plant.

TABLE II

NO_3^- uptake determined by ^{15}N labelling, and shoot N concentration of Chrysanthemum x morifolium *after 3 weeks growth at a day temperature of 20ºC and 4 combinations of night air temperature (NAT) and night root zone temperature (NRT). All values, except shoot N content are calculated on the basis of plant dry weight, after the subtraction of starch from the leaves.*

Temperature		NO_3^- uptake (night)	NO_3^- uptake (day)	Shoot N	Shoot N
NAT	NRT	(mg g^{-1} h^{-1})	(mg g^{-1} h^{-1})	(mg per shoot)	(mg g^{-1})
8	8	0.16[a]	0.94	591.1	56.12
	20	0.40	0.81	665.1	53.52
20	8	0.24	0.83	610.6	60.61
	20	0.39	0.75	684.5	58.00
Factors	df	*P* value[b]			
Harvest	3	**	**		
NAT	1				**
NRT	1			*	

[a]Each value is the mean of three replicates.
[b]*P* value for differences of means (Linear mixed-effects model).
** significant at $P < 0.01$.

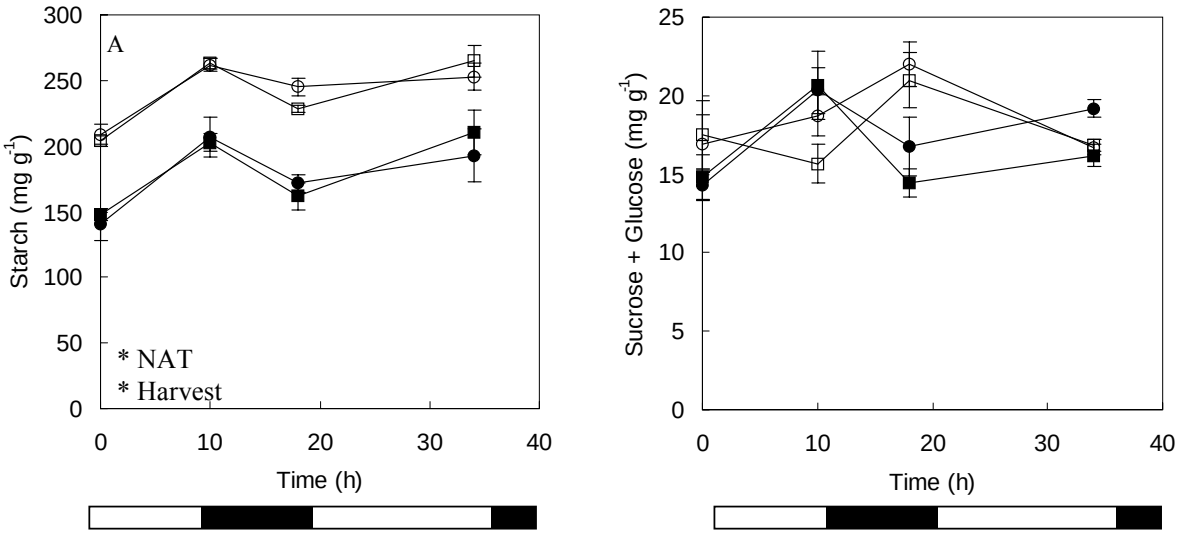

FIG. 4

Diurnal variation in carbohydrate concentrations in leaves of *Chrysanthemum x morifolium* after 3 weeks growth at a day temperature of 20ºC and 4 combinations of night air temperature (NAT) and night root zone temperature (NRT). NAT of 20ºC (black symbols) or 8ºC (white symbols). NRT of 20ºC (squares) or 8ºC (circles). The black and white bar in bottom of the figure illustrates the dark and light period. Starch concentration in leaves (A). Concentration of sucrose and glucose in leaves (B). (n = 3, * $P < 0.05$).

DISCUSSION

The results show, that the benefits of root zone heating on growth and physiological processes are limited in *Chrysanthemum x morifolium*, at least, when plants are grown at 8ºC NAT. Plant biomass is unchanged, which confirms the results found in other studies on chrysanthemums grown at low night temperature with root zone heating (Brown and Ormrod, 1980; Tsujita *et al*., 1981). However, plants grown at 8ºC NAT with root zone heating are still restricted by sink limitation during the night as starch accumulated in the leaves due to a decrease in phloem loading at low air temperature. The NAT needs to be optimised for this process, if plants are to benefit from the increased NRT.

Diurnal changes in carbohydrate levels and starch accumulation.

Starch accumulated in the leaves at 8ºC NAT, and the diurnal changes in starch levels were not changed by differences in NAT. The results are contradictory to results from a former study, where starch synthesised during the day, did not disappear during the dark period (Kjær *et al*., accepted). It is suggested, that the final level of starch accumulation of a certain leaf may be reached in a few days, and that the leaves were fully starch-saturated at the sampling date in the present experiment. However, more work is needed to confirm this relationship.

NRT did not decrease starch content in the leaves and had no effect on the diurnal variation of soluble sugars (sucrose and glucose) or the starch in the leaves, which suggests that the regulation of the carbohydrate metabolism is locally, regulated by the temperature of the leaves and not by the time a day, or the carbohydrate demand of the roots. It is a well known phenomenon, that low temperatures increase the amount of starch and soluble sugars in plant tissue (Gamalei *et al*., 1994; Hurry *et al*., 1995).

Starch accumulation, N-content and photosynthetic capacity

A negative linear correlation between starch accumulation in the four fully expanded leaves and the approximated maximum capacity for CO_2 assimilation, over the time span of 3 weeks confirmed, that a decrease in CO_2 assimilation are related to an increased accumulation of starch in leaves (Goldschmidt and Huber, 1992). However, the relation was not consistent, as 20ºC NRT at 8°C NAT increased CO_2 assimilation, without decreasing the starch accumulation in the leaves. It is suggested, that the N-content of the shoots, which were increased by root zone heating may have contributed to the increase in the maximum photosynthetic capacity in this treatment, as a positive relation between leaf N content per leaf area and photosynthetic capacity is well-known (Evans, 1989).

The NO_3^- uptake

It was demonstrated, that differences in NRT and NAT did not affect the total daily NO_3^- uptake. Although, the results indicated that the NO_3^- uptake rate during the night was reduced at 8ºC NRT, and increased during the day, which further suggested that plants grown at 8ºC NRT, compensated by having high NO_3^- uptake rates during the day. A potential decrease in NO_3^- uptake at 8°C NRT during the night was expected, due to other results on nutrient uptake and water relations of plants grown at different root zone temperatures (Bassirirad, 2000; Castle *et al*., 2006).

The earlier reported decrease in shoot N concentration of *Chrysanthemum x morifolium* grown at LNT (Kjær *et al*., accepted), was confirmed in this study. A decrease in plant N concentration is closely related to increased starch accumulation and decreased photosynthesis (Geiger *et al*., 1999; Paul and Foyer, 2001). In contrast, the shoot N content increased at 20ºC NRT at 8ºC NAT in the present study, possibly as a effect of increased shoot DW in this treatment, although this was not seen in the plant morphology results when measured across 3 weeks. The increase in shoot N content may be related to the increased maximum capacity of photosynthesis in this treatment

Only few studies are published, concerning the effect of plant responses to differences in NRT and NAT. The present study indicates that the temperature difference caused and imbalance in the sink-source balance of the plants, which changed carbohydrate metabolism, nutrient uptake and plant morphology. In the present experiment it is shown that root zone heating of *Chrysanthemum x morifolium* grown at 8ºC NAT, did not increase the root activity in a way, that affect the flow of carbohydrates from the source leaves. Instead, carbohydrates remained in the leaves as starch, possibly because of restrictions in phloem loading (Gamalei *et al*., 1995).

8ºC NAT is regarded a very low NAT for production of chrysanthemums, and it is suggested that a positive effect of NRT may be present, if the NAT is not limiting plant growth (Gosselin and Trudel, 1986, Thompson *et al*., 1998). Also, it is suggested, that optimised NRT will increase the ability of the plants to adapt to the high day temperatures, which can be obtained, in the dynamic climate control system (Aaslyng *et al*., 2003).

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Paper III

Starch Accumulation in Leaves of *Chrysanthemum x morifolium* at Low Night Temperatures Depend on the CO_2 concentration

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Starch Accumulation in Leaves of *Chrysanthemum x morifolium* at Low Night Temperatures Depend on the CO_2 concentration

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Abstract. A greenhouse study was performed in the autumn of 2006 and replicated in the winter of 2007 to study the effect of low night temperature (LNT) on plant growth responses in vegetative *Chrysanthemum x morifolium*. Plants were grown under long day conditions in four climates with a photoperiod of 17 hours. The four climates had a day temperature set point of 22ºC, and four combinations of night temperatures and CO_2 concentrations during the day, when vents were closed; The night temperature set points were 20ºC and 12ºC, and set points of CO_2 concentrations were, ambient [CO_2] 350 $\mu l \cdot l^{-1}$ and High [CO_2] 900 $\mu l \cdot l^{-1}$. The set point of 12ºC night was not reached at any time in the autumn experiment, and not before 2 hours after the start of the dark period in the winter experiment. However, the LNT set point of 12ºC increased starch accumulation in the leaves in both experiments, although the increase was much more pronounced at high CO_2 concentrations in the winter experiment, compared with the autumn experiment. The set point of 12ºC night had no significant effect on plant dry matter (DM) production or NO_3^- uptake, but 12ºC night reduced stem length in the winter experiment. High [CO_2] increased shoot DM, leaf DM, the number of leaves and stem length. The present results indicate that starch accumulation as a response of chrysanthemum to LNT depend on contributing factors, such as [CO_2] and light intensity. Furthermore, it is concluded, that it is possible to grow chrysanthemums with a night temperature set point of 12ºC, without a loss in DM production and significant changes in morphology.

Dynamic climate control is a system which optimises greenhouse production of plants in order to save energy. Heat and CO_2 is supplied in periods with high irradiance and saved during the night and in periods with low irradiance (Aaslyng *et al*., 2003). Plant production is generally maintained at the same level as in more "traditional" systems, which tend to keep temperature as constant as possible; however, there are exceptions. The time to flowering may increase, and plant quality may decrease, if the night temperature, and thereby also the mean temperature, is too low, (Lund *et al*. 2006; Ottosen *et al*., 2003). Recently, it was shown that a low night temperature (LNT) below 12ºC increase starch accumulation in the upper-most fully expanded leaves of *Chrysanthemum x morifolium* when grown under controlled climate chamber conditions and in nutrient solution culture (Kjær *et al*., accepted). LNT did not significantly affect plant DM production or the daily NO_3^- uptake; however, plants had fewer leaves and a smaller leaf area. The results indicated that LNT caused sink limitations to the plants, because of a temperature-induced inhibition of phloem loading in the leaves (Gamalei *et al*., 1994). The carbohydrates remained in the leaves as starch on the expense of an investment in leaf initiation and expansion.

It is hypothesised in the present study, that plant responses seen in former climate chamber studies, may no be present at the same magnitude in the greenhouse, because fluctuations in temperature and irradiance from the sun, and high CO_2 concentrations, which is a common strategy in optimising plant growth and photosynthesis at high irradiance in greenhouse production, may override the negative effect of the LNT. However, although high [CO_2] increase growth in good light conditions (Mortensen and Moe, 1992; Ainsworth and Long, 2005), high [CO_2] may as well contribute to the starch accumulation in the leaves (Katny *et al*., 2005)

When plants are grown at a large positive difference between day and night temperature they are often taller, than if the difference between day and night temperatures is small. The concept is known as positive DIF and is well-known in many plants, including chrysanthemum (Myster and Moe, 1995). However, when plants of *Hibiscus rosa-sinensis* where grown in a dynamic climate system with a more positive DIF, than in a more traditional climate, the plants became shorter (Lund *et al*., 2006). In the particular experiment, the mean temperature difference between day and night was not reflected in the temperature difference of individual days, because warm days were often followed by warm nights and vice versa, and this was explained as one possible reason for the missing increase in stem length.

The aim of the present study was to clarify, whether plant responses to LNT seen in climate chamber experiments are present, when plants of *Chrysanthemum x morifolium* are grown in a greenhouse, or whether the plant responses are limited due to, the not so precise climate obtained in the greenhouse. Furthermore, plants were grown at two different CO_2 concentrations to study whether high [CO_2] may have a contributing effect on the effect of LNT. Plants were kept vegetative in the experiments, to confirm that a possible starch accumulation at a LNT in greenhouse production of pot plants, has only limited effect on plant growth in terms of DM production. This may confirm the possibility to use lower temperatures during the long day treatment of chrysanthemums and other pot plants in production, than formerly used.

Materials and Methods

Experimental design. Cuttings of *Chrysanthemum x morifolium* cultivar 'Coral Charm' were rooted in pots (11 cm in diameter). After 3 weeks, plants were pinched back to three leaves, and grown for 7 weeks in a long day treatment in a greenhouse. Two replicate experiments were carried out in four similar compartments of 9.9 x 7.6 m in a greenhouse located at The Department of Horticulture, Aarhus University (Aarslev, Denmark, lat. 55°N). Each compartment was equipped with four tables (each 9.75 m^2), but the chrysanthemum experiment was only located on one table and the rest of the tables were occupied by plants of other experiments. The first experiment was carried out from September 11 to October 29, 2006 (autumn experiment) and the second experiment was carried out from January 3 to February 20, 2007 (winter experiment). The set points of the four treatments were a day temperature of 22ºC and four combinations of night air temperature and CO_2 concentrations during the day: Night temperatures were 20ºC (NNT) and 12ºC (LNT). The CO_2 concentrations were 900 $\mu l \cdot l^{-1}$ (High [CO_2]) and 350 $\mu l \cdot l^{-1}$ (Ambient [CO_2]). Each treatment had three replicates. The photoperiod was 17 h, from 07:00 h to 24:00 h. Supplementary light (High-presure sodium lamps SONT-T agro, 600 W, Phillips, The Netherlands) provided 32 ± 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the top of the plant canopy during the whole photoperiod, whenever the photosynthetic flux density (PPFD) fell below 198 $\mu mol \cdot m^{-2} \cdot s^{-1}$ outside the greenhouse. The heat was turned on during the beginning of the photoperiod, and the vents were open during the beginning of the dark period to reach the temperature set points of night and day within one hour. Plants were fertigated by flooding of the tables for 20 min every second day, with nutrient solution consisting of: 5.7 mM $NaNO_3$, 12.6 mM NH_4NO_3, 8.9 mM KCl, 0.7 mM Fe-EDTA, 47.6 mM KNO_3, 0.4 mM $(NH_4)^2SO_4$, 3.4 mM $(NH_4)H_2PO_4$, 2.6 mM KH_2PO_4, 5.8 mM $MgSO_4$ and micronutrients.

Throughout the experiment, the air temperature, relative humidity (RH), and CO_2 concentrations were logged with 1 min intervals in each greenhouse compartment just above plant canopy using a standard environmental control software (Completa, Senmatic, Denmark), it was further logged, when the supplementary light was turned on or off, and the position of the vents. On the roof of the greenhouse, a weather station logged global irradiance, the air temperature, wind speed and direction.

Plant growth. The pinched plants developed three lateral branches, which were all harvested from each plant for growth analysis a three time-points during each experiment. In the autumn experiment, three plants from each replicate were harvested to constitute a total of nine plants from each treatment after 5, 6, and 7 weeks. In the winter experiment the same amount were harvested after 3, 4, and 7 weeks. Each harvest began just before the start of the photoperiod, where leaf samples were collected for carbohydrate analysis. Thereafter, stem length was measured, as the total length of the first lateral branch. Leaves were separated from all three lateral branches and counted, and leaf areas were measured on a LI-COR portable leaf area meter (LI-3000, Lambda Inst. Corp., United States). Fresh weight (FW) of leaf and stem material was determined, before drying at 70ºC for 24 h for determination of dry weight (DW).

Carbohydrate analysis. At each harvest, the four upper-most fully expanded leaves were sampled from three plants, in each replicate, in each treatment, and directly frozen in liquid nitrogen and stored at -80ºC. The leaf samples were sampled *in situ*, when the plants were still located in the greenhouse. Because of this sampling method, there was a time-difference

between the time at which the first sample was collected and freezed in liquid nitrogen, and the samples from the second and third replicate, 15 min and 30 min, respectively.

Prior to analysis, samples were freeze-dried and ground in liquid nitrogen. Concentrations of hexose (glucose + fructose) and sucrose were determined on a HPLC (Hewlett Pacard 1047A, Waldbronn, Germany) as described by Liu *et al*. (2004). Starch was determined in the remaining pellets after extraction of soluble sugars as described in Kjær *et al*. (accepted).

Overnight uptake of $^{15}NO_3^-$. An overnight analysis of $^{15}NO_3^-$ uptake in the plants was performed at the first harvest, after 3 weeks growth in the winter experiment. Plants were kept dry for two days, before the start of the analysis, to assure that the soil could absorb the nutrient solution, when applied. 200 ml of a nutrient solution, containing 3.4 atom% $^{15}NO_3^-$, was applied to saucers, placed beneath the pots of a total of six plants in each replicate of two treatments, 1 h before the dark period. The two treatments were High [CO_2], NNT, and High [CO_2], LNT. Three randomly chosen plants were harvested at the same time (1 h before the dark period) to constitute the control plants. Two plants from each replicate, in each treatment, were harvested after 1 h light (right before the dark period), 7 h dark, and after 15 h light the following day to provide a total of three harvest times, and the control. At each harvest, shoot FW was determined, before drying at 70ºC for 24 h for determination of DW.

The dried shoot material was finely ground (< 0.25 mm) and analysed for ^{15}N and total-N by elemental analyser isotope ratio mass spectrometry (Europa Scientific 20-20 IRMS) in a commercial laboratory (Iso-Analytical ltd., United Kingdom). Excess ^{15}N enrichment of plant material after the dark period, and after the following light period, were calculated by subtraction of natural ^{15}N enrichment of plant material determined in the control plants.

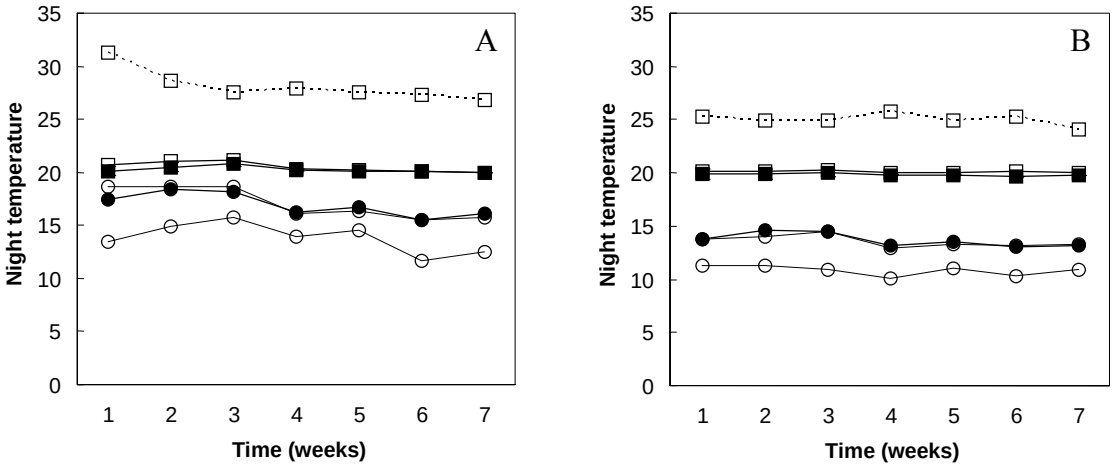

Fig.1. The average night temperature (ANT, filled lines) in each of four treatments in four similar greenhouse compartments in two experiments carried out during autumn (A, 11.09.06-29.10.06) and winter (B, 03.01.07-20.02.07). Set points of the treatments were: High [CO_2] (black), ambient [CO_2] (white). Night temperature set points of 20°C (squares) and 12°C (circles). Average minimum night temperature in the 12ºC night temperature treatments (circles, dashed line). Average maximum day temperature in all treatments (squares, dashed line). (The data are the average for each of the 7 weeks, in each experiment. Data were collected with 1 min intervals).

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Data analysis. Means, maximums, sums and standard errors of the PPFD in each of three periods in each experiment was analysed using SAS statistical software (SAS institute, V8.02, 1999). Furthermore, differences in the status of supplementary light, temperature, relative humidity (RH), and CO_2 concentrations between treatments, periods and experiments were also analysed in SAS. Differences across treatments in plant morphology, $^{15}NO_3^-$ uptake and the distribution of non-structural carbohydrates were analysed by the linear mixed effects model allowing for nested random effects using software package R release 2.4.1 (http://w-ww.r-project.org).

Results

Temperature, irradiance and CO_2 concentrations in the greenhouse. The average day temperature (ADT) measured on the basis of results from the climate control software was similar in all treatments in each experiment, 23°C in the autumn experiment and 22°C in the winter experiment (results not shown). The night temperature set point of 12°C was, on average, not reached in the autumn experiment. The average night temperature (ANT) was between 15.5ºC – 18.7ºC (Figure 1A). In the winter experiment the night temperature set point of 12°C was reached after 2 hours dark, and the average night temperature (ANT) was approximately 13.1°C – 14.6ºC (Figure 1B). The light integral of the photosynthetic flux density (PPFD, µmol m^{-2} s^{-1}) was recalculated from the global irradiance outside the greenhouse. In the winter experiment it was much lower, than in the autumn experiment (Figure 2A). More supplementary light was provided during the winter experiment, however this did not compensate for the low irradiance in the winter experiment in comparison with the autumn experiment (results not shown).

The average irradiance was lower during all weeks in the winter experiment in comparison with autumn experiment. However, the values decreased in the autumn and increased in the winter, due to the decrease and increase in day length, respectively. The mean irradiance was close to similar during the last 2 weeks in the two experiments (Figure 2B). The high CO_2

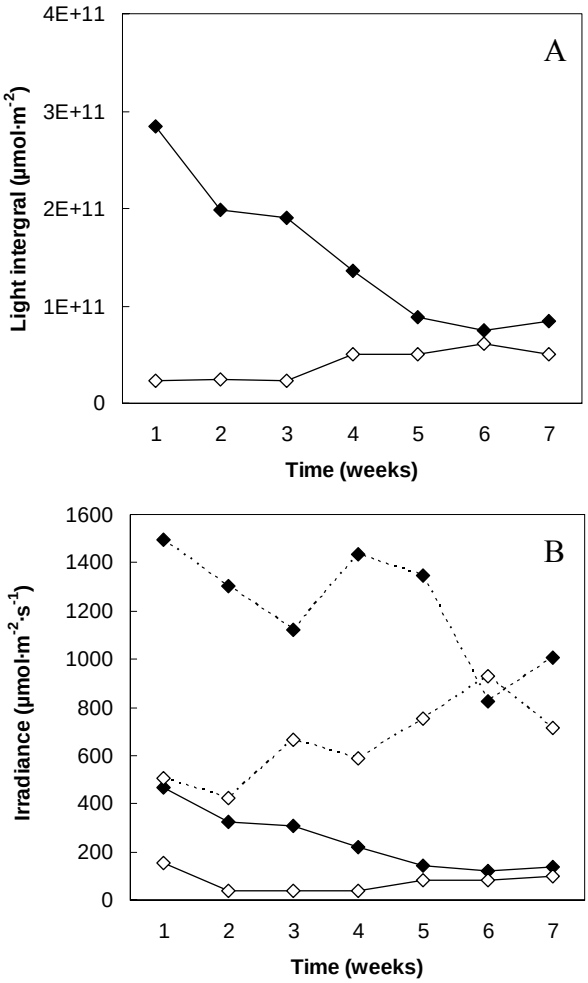

Fig. 2. (A) The Light intergrals in two experiments carried out in during 7 weeks in the autumn (11.09.06-29.10.06) (black diamonds), and 7 weeks in the winter (03.01.07-20.02.07) (white diamonds).
(B) Average irradiance (filled lines) and max irradiance (dashed lines) measured outside the greenhouse in 7 weeks of the two experiments; autumn experiment (black diamonds) and winter experiment (white diamonds).

concentration of 900 μl·l^{-1} was on average not reached in the autumn experiment and the average difference between the two [CO_2] treatments was below 300 μl l^{-1} in the autumn experiment and not different and above 400 μl·l^{-1} in the winter experiment and different (Figure 3A and B).

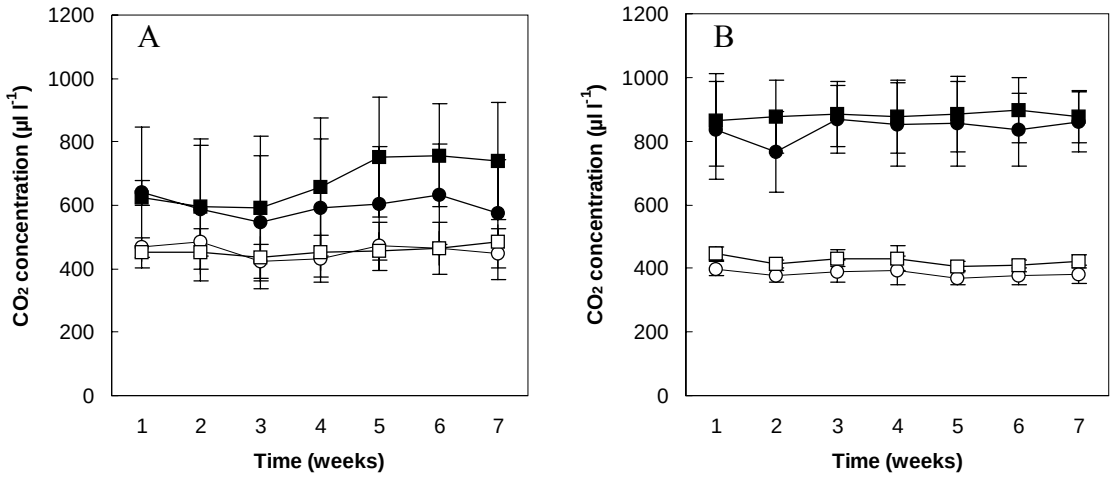

Fig.3. The CO_2 concentrations during the day in each of four treatments in four similar greenhouse compartments in two experiments carried out during autumn (A, 11.09.06-29.10.06) and winter (B, 03.01.07-20.02.07). Set points of the treatments were: High [CO_2] (black), ambient [CO_2] (white). Night temperature of 20°C (squares) and 12°C (circles). (The data are the average for each of the 7 weeks in each experiment ± SE of each week, data were collected with 1 min intervals).

Plant growth and morphology. Plants accumulated more DM in the winter experiment in comparison with the autumn experiment (Figure 4A). Furthermore, the RGR was 0.10 g·g^{-1}·day $^{-1}$ ± 0.01 during the last two weeks in the winter experiment, whereas the RGR was only 0.06 g·g^{-1}·day $^{-1}$ ± 0.02 during the last three weeks in the autumn experiment (no differences were found between treatments in each experiment, results not shown).

In contrast, plants in the autumn experiment were taller than plants in the winter experiment (Figure 4B). Generally there was no effect of night temperature and CO_2 concentrations on any of the morphological parameters in the autumn experiment; whereas, the low night temperature set point of 12ºC (LNT) reduced stem length and high [CO_2] increased stem length, shoot DM, leaf DM and leaf number in the winter experiment (Figure 4A and B, results not shown for leaf DM and leaf number).

Long term changes in carbohydrate levels. The carbohydrate content of the upper-most fully expanded leaves was analysed after 7 weeks in the autumn experiment and after 4 and 7 weeks in the winter experiment. LNT significantly increased starch content in the leaves in both experiments (Figure 5A and B). After 7 weeks, the leaf starch content at LNT was more than 100% higher in plants grown in the treatment with high [CO_2] in the winter experiment, in comparison with leaf starch content at LNT, in plants grown in the same treatment, in the autumn experiment.

High [CO_2] also increased starch content in the leaves, but the increase was only significant in the winter experiment after 7 weeks growth. The effect of LNT on the concentration of solu-

ble sugars differed between the experiments. In the autumn experiment, high [CO_2] slightly increased the concentrations (Figure 4D). In contrast, LNT increased the concentration of soluble sugars in the leaves of chrysanthemum at both harvests in the winter experiment, and the CO_2 concentrations had no effect (Figure 4C and D).

NO_3^- uptake. Neither LNT or High [CO_2] had any effect on the N concentration or the N content of the shoots of *Chrysanthemum x morifolium* (results not shown). The mean excess atom% of ^{15}N in the control plants harvested before the addition of $^{15}NO_3^-$ was 0.3674 and close to the naturally occurring atom% of ^{15}N (0.3663%). After 1 h light and 7 h dark, all plants had taken up $^{15}NO_3^-$, but no significant differences were found between the two treatments, and after the following 15 h light, there we-re still no significant differences in the excess ^{15}N in the plant shoots between treatments (results not shown). The NO_3^- uptake rate during the night was lower in plants grown at LNT; however, significant differences in total NO_3^- up-take rate were only seen between night and day, and not between treatments. The NO_3^- uptake during the night was 30% of the NO_3^- rate during the day (results not shown).

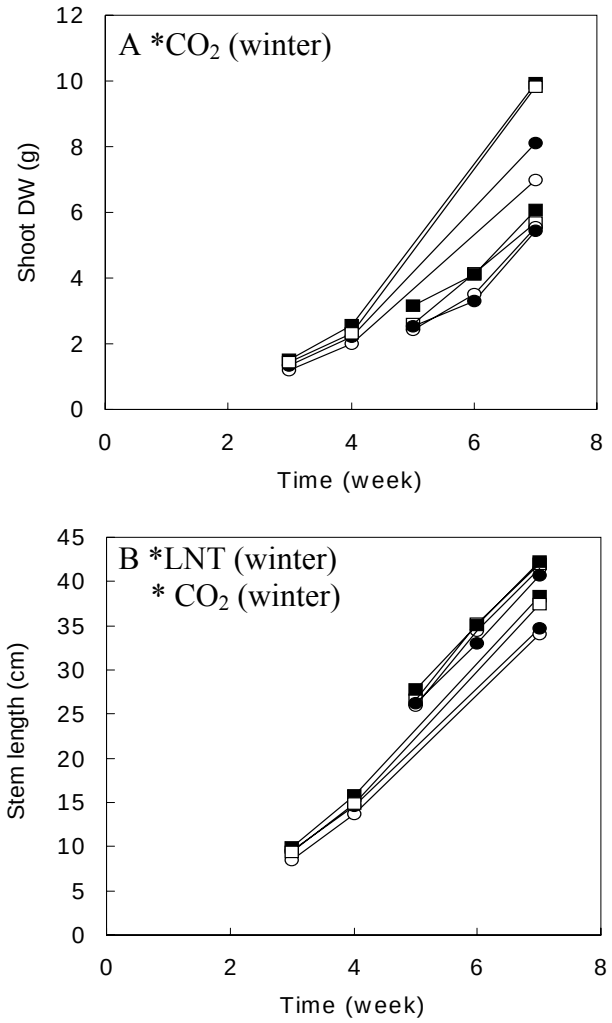

Fig. 3. Shoot dry matter (DW) and stem length of *Chrysanthemum x morifolium* grown at a day temperature set point of 22ºC, and four com-binations of night temperature and [CO_2] during the day. Night temperature of 20ºC (Squares) or 12ºC (circles), respectively. Ambient [CO_2] of 350 µl·l^{-1} (white) and high [CO_2] of 900 µl·l^{-1} (black). Results from both experiments are shown in each figure. Plants were harvested after 5, 6 and 7 weeks in the autumn experiment, and after 3, 4 and 7 weeks in the winter experiment.
(n = 9, * P < 0.05).

Discussion

In the present experiments, it was demonstrated, that LNT affect carbohydrate metabolism of the upper-most fully expanded leaves, when plants are grown under greenhouse conditions, in much the same way, as when they are grown in climate chambers. This was found, even though, higher night temperatures were used, than in the earlier climate chamber experiments (Kjær *et al.*, accepted).

Furhermore, there was a difference between the autumn and winter experiments due to the unusually high outdoor temperatures during the autumn, which made it impossible to cool the greenhouse down to 12ºC during most

of the nights. The high outdoor temperature during the autumn also resulted in lower CO_2 concentration in the high [CO_2] treatment due to a longer time of ventilation to obtain the required day temperature.

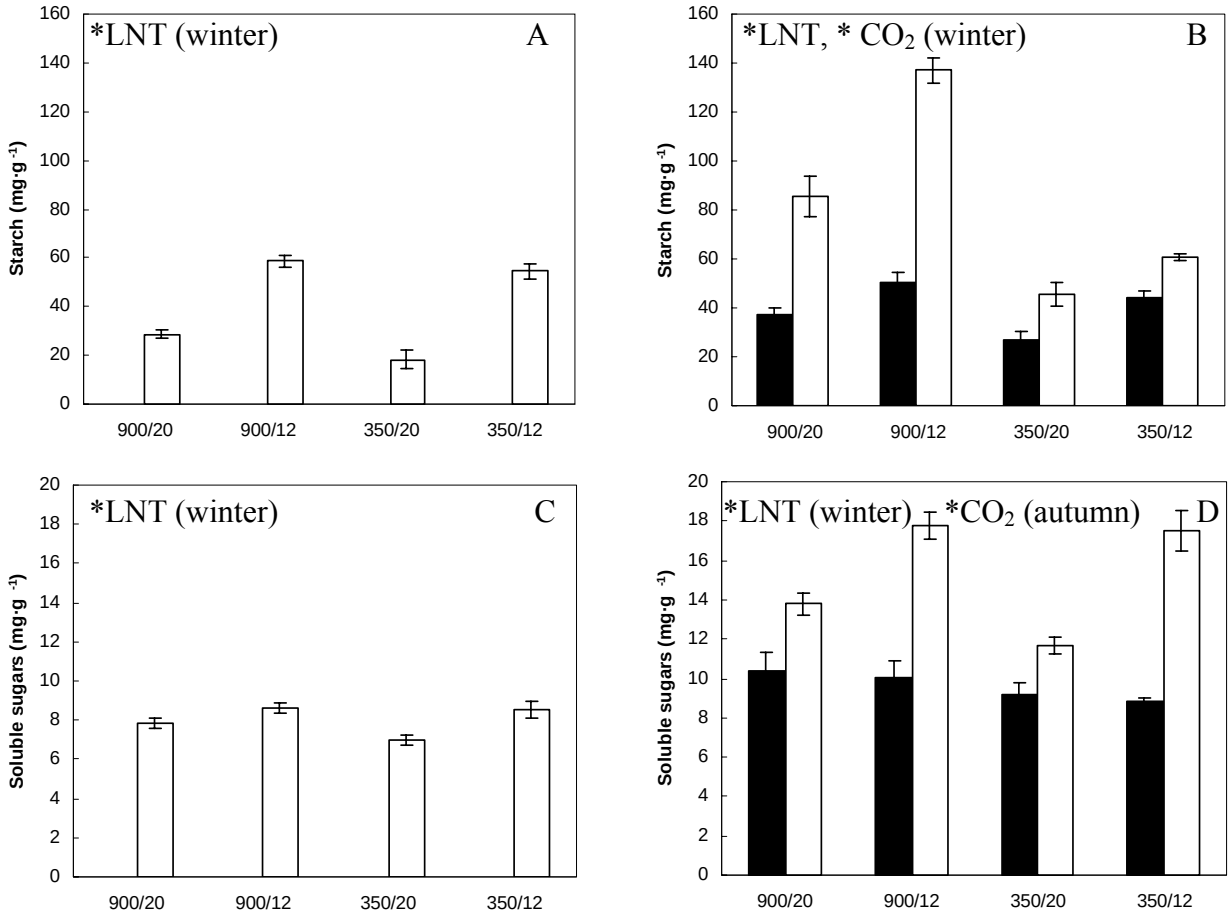

Fig. 4. Carbohydrate concentrations in the upper-most fully expanded leaves of *Chrysan-themum x morifolium* after 7 weeks in autumn experiment (black coloumns) and after 4 and 7 weeks in the winter experiment (white columns), at a day temperature of 22ºC and 4 combinations of night temperature and [CO_2] during the day. Night temperature set points of 20ºC (20) or 12ºC (12), respectively. CO_2 of 350 $\mu l \cdot l^{-1}$ (350) or 900 $\mu l \cdot l^{-1}$ (900), respectively. Starch concentrations after 4 weeks (A), and 7 weeks (B). Concentrations of soluble sugars including sucrose, glucose and fructose after 4 weeks (C), and 7 weeks (D). (n = 6, $*P < 0.05$).

Leaf content of starch and sugars. The leaf starch content was higher in the winter experiment and responded to both LNT and high [CO_2], in comparison with the autumn experiment where the leaf starch content only responded to LNT. In the autumn experiment the night temperature set point of 12ºC was not reached, and the ANT was more than 2ºC higher during most of the weeks, compared to the winter experiment. Furthermore, the difference in CO_2 concentrations between the two [CO_2] treatments was much lower in the autumn experiment compared to the winter experiment. This smaller difference in [CO_2] concentrations between treatments, and the warmer temperatures in the autumn experiment may explain why the effect of LNT and [CO_2] was much lower in this experiment compared to the winter experiment. The

difference in light intensities between the two experiments were not expected to contribute to the effect of LNT and high [CO_2] on the leaf starch content, as Kuehny *et al*. (1991) showed that the leaf starch content increased in response to an increase in CO_2 concentration and that a difference in light intensities, ranging from 830 µmol·m^{-2}·s^{-1}, to 1400 µmol·m^{-2}·s^{-1} had no effect on this relationship.

The leaf content of soluble sugars also responded differently to temperature in the two experiments. It is known from the literature that low temperatures increase leaf content of sugars (Hurry *et al*., 1995), and it is suggested that the night temperature in the autumn experiment were not low enough to show this relation.

Starch accumulation and plant growth. Starch accumulation did not affect plant growth and morphology in any of the treatments. The higher RGR during the last two weeks in the winter experiment was probably caused by the increase in day length in contrast to a decrease in day length in the autumn experiment. The increased DM content of shoots and leaves at high [CO_2], and the decrease in stem length at LNT of plants in the winter experiment was probably an effect of the temperature and [CO_2] conditions being more pronounced as described earlier.

The shorter stem length at a positive DIF of approximately 8ºC in comparison with 2ºC in the winter experiment was in contrast to results from the literature (Myster and Moe, 1995). In the present experiment, the decrease in stem length may not be explained by large fluctuations in day temperatures, as suggested by Lund *et al*. (2006); because temperature fluctuations were similar in all treatments of the present experiments. Instead, it is be suggested that the increased leaf starch content in the present experiment, decreased the supply of carbohydrates available for stem elongation, as also shown in a study by Kaufmann *et al*. (2000). However, in that study, it was suggested that the limitations to stem growth occurred, because the carbohydrates were used in root growth. In the present study, it is suggested that the supply of carbohydrates for stem growth was decreased because the carbohydrates remained in the leaves as starch, as temperatures below 12ºC, have been shown to limit the carbohydrate export from the leaves (Kjær *et al*., accepted). The positive effect on stem length at a positive DIF in cucumber can be reduced by lowering the night temperature only (Grimstad and Frimandslund, 1993)

Starch accumulation and growth environment. The leaf starch content in plants, grown for 7 weeks in the treatment with ambient [CO_2] and with an approximately average night temperature of 13.1ºC – 14.6ºC was only 38% of the leaf starch accumulation at an average night air temperature of 12.2°C in a climate chamber treatment where plants were also grown at ambient [CO_2] (350 µl·l^{-1} CO_2) (Kjær *et al*., accepted). Comparing these results suggest, that differences in the climatic environment of climate chambers and greenhouses do have an influence on the carbohydrate metabolism of plants. One explanation may be that the average night temperature in the greenhouse was higher. Another explanation may be that the fluctuations in light and temperature of the greenhouse environment interrupt the well-known diurnal fluctuations in starch synthesis and breakdown, which occur in the leaves of many plants, when grown under climate chamber conditions (Stitt *et al*., 1978). The fluctuations may cause some starch to be degraded during the photoperiod, although until now, starch breakdown is mainly thought to occur during the night, and to be regulated by the length of the preceding photoperiod (Zeeman *et al*., 2007). Fondy et al. (1989) demonstrated, in a study on bean and sugar beat, that starch degradation occurred when the photosynthesis was below a threshold rate, approximately 200 µmol m^{-2} s^{-1} for sugar beat and 400 µmol m^{-2} s^{-1} for bean, and starch accumulation occurred

above this rate. Degradation of starch at low irradiance levels may explain why the increase in starch content was much lower in the greenhouse climate compared to the climate chamber where the light level was constant throughout the light period, and above 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

The NO_3^- uptake. Shoot N content and shoot N concentration was not decreased by LNT at increased CO_2 concentration, which demonstrated that plants were not limited by N. The NO_3^- uptake during the night constituted for 30% of the daily uptake, and no differences were seen between treatments, which confirmed earlier work on chrysanthemums grown in nutrient solutions (Kjær *et al*., accepted). Furthermore, no significant difference was seen in the uptake rate during the night and day. Low soil temperature is known to decrease nutrient transport and root growth in soil (Tinker and Nye, 2000; McMichael and Burke, 1998). However, in the present study it was shown, that these general effects, did not significantly affect NO_3^- uptake when plants were grown in small pots (11 cm).

It is concluded from the present experiments, that vegetative growth of *Chrysanthemum x morifolium* cv. "Choral Charm" in a greenhouse climate, where the night temperature is allowed to drop to 12ºC, is possible, without major limitations in growth and morphology. Starch is accumulated in the leaves because of increased [CO_2] and as a restriction of sink limitation during the night, which may contribute to less carbohydrate available for stem growth. The results suggest that it is possible to produce chrysanthemums at reduced night temperatures during the long day treatment, which main purpose is to generate DM production of the plants. However, concerns need to be taken in choosing the [CO_2] concentration for the production. Furthermore, more work is needed to resolve the effects of low levels of light intensity on starch accumulation and degradation.

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